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"LITTLE CHERRY," A VIRUS DISEASE[1]

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In July, 1940, the senior author's attention was directed to a cherry disorder which was present in the Kootenay Lake area of British Columbia, and which was reported by E. C. Hunt, District Horticulturist, to have spread considerably during 1939. The seriousness of this disease is largely due to the great rapidity with which it can spread to every tree in an orchard and from one orchard to another. Information received from several sources indicates that the disease first appeared in an orchard on the West Arm of Kootenay Lake in 1933. Extensive investigations by the senior author show that the disease has now spread about 20 miles north, at least 15 miles west, and has crossed the main lake and reached Creston 45 miles south-east. In a number of districts, 100% of the trees are affected. The cherries from affected trees are not usually suitable for the fresh fruit trade, but can be used for processing purposes. The object of this paper is to present the results of transmission experiments which established the virus nature of the disease.

SYMPTOMS

The name "little cherry," which is in popular use, indicates the most striking symptom of the disease. The fruits on affected trees are about half the size of those on healthy trees grown under the same conditions (Figure 1). The average volume of Lambert cherries in one orchard in 1945 was, from affected trees, 3.5 cc., and from healthy trees, 6.5 cc. The average diameter of affected and healthy fruits in the same orchard was 17 mm. and 23 mm., respectively. The difference in size begins to appear about 2 weeks before maturity and the maximum difference occurs when the fruit on healthy trees is ripe. Affected fruits, particularly in the Lambert variety, are inclined to be more angular and pointed than normal, and often have 3 nearly flat sides tapering towards the distal end (Figure 1). Nearly all affected Lambert fruits retain the bright red of an immature cherry far beyond the normal picking date. This bright colour is very pronounced in the Republican variety. Ripe affected fruits do not have the sweetness and flavour of healthy cherries. Sometimes a few fruits are normal in size and colour, but lack quality. Diseased trees set and retain large numbers of fruits, and at picking time much of the crop remains readily visible above the leaves, whereas in healthy trees the cherries are largely hidden under the leaves.

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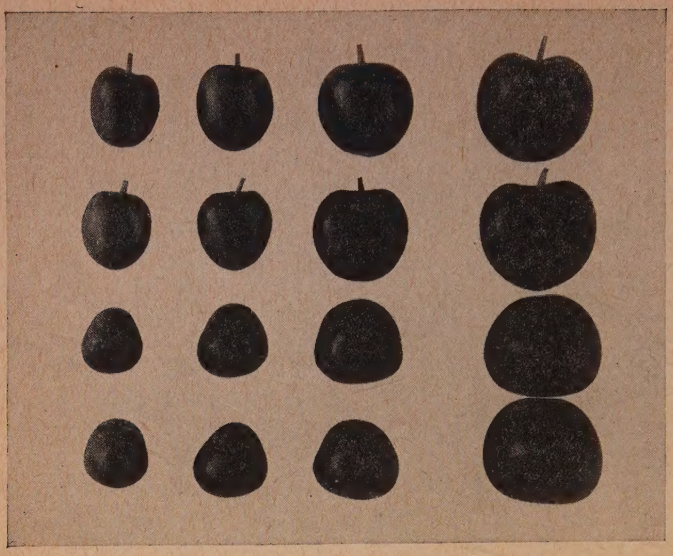

FIGURE 1. Fruit of the Lambeth cherry variety: the left 3 rows are affected with "Little Cherry"; the row on the right is healthy.

The growth of affected trees appears to be normal, uniform, and vigorous, unless the orchard is neglected. The leaves are not known to show any symptoms which may not be found in trees not affected with this disease. The severity of the disease varies a little from year to year and quite considerably in different varieties. The Lambert, a severely affected variety, seldom has any apparently normal fruits after it has been affected for a number of years, but the Bing, a moderately affected variety, may have from year to year a varying amount of fruit suitable for the fresh fruit trade.

TRANSMISSION EXPERIMENTS

On July 31, 1940, buds from Lambert cherry trees, affected with "little cherry," from Willow Point in the Kootenays, were put on 3 healthy Lambert cherry trees and on 10 Mazzard seedlings on the Experimental Station at Saanichton. Most of the fruits on 2 of the budded trees showed typical symptoms of "little cherry" on July 4, 1941. The number of fruits on the other budded tree was insufficient to make a diagnosis possible. The average maximum diameter in millimeters of 200 fruits, 100 from each of the affected trees, was $17.6 \pm .23$, while a similar number from 2 healthy check trees was $21.2 \pm .34$, a difference of 3.6 mm. While the difference in size of the fruits of "little cherry" and healthy check is easily significant, it probably would have been greater still if it had not been necessary to take the measurements about 4 days earlier than desirable, i.e., before maturity, on account of attack by birds. The transmission of the "little cherry" disease to healthy trees by budding indicates that it is caused by a virus. The budded Mazzard seedlings showed no recognizable symptoms (2).

For further and more adequate transmission tests, a well isolated and healthy orchard at Kootenay Bay was located by E. C. Hunt, who also assumed responsibility for its horticultural care and maintenance. The

orchard was provided by the Provincial Department of Agriculture and the pathological work was done by the Dominion Laboratory of Plant Pathology, Summerland, B.C. The majority of the 60 trees were of the desirable Lambert and Republican varieties, and bore sufficient fruit to make diagnosis possible. In 1943, buds from affected trees in the J. D. McDonnell orchard at Willow Point were put on 24 of these healthy trees. In order to test as many diseased trees as possible, each healthy tree received buds from a different diseased tree.

In 1943, 16 Lambert trees received diseased Lambert buds. In 1944, 12 of them showed definite symptoms of "little cherry," 3 were highly suspicious, and 1 appeared healthy, while 10 untreated Lambert check trees remained healthy. In 1943, 4 Republican trees were budded with diseased Republican buds. In 1944, they all showed definite symptoms of "little cherry" but 4 untreated Republican check trees remained healthy. The other 4 budded trees did not have crop enough to give results. In 1945, every bearing tree throughout the main central part of the orchard was definitely affected. Only 4 trees at the extreme edge of the orchard remained apparently free from "little cherry." In general, the trees that had "little cherry" for the second year were affected throughout, while, as in 1944, trees that were showing the disease for the first time were only partly affected.

The average volume of 100 fruits from each of 3 healthy Lambert trees in 1944 was 8.1 cc. That of fruits from the same trees in 1945, after they had become naturally infected, was 3.9 cc. The average percentage of soluble solids in 30 fruits from each of the same trees was 17.5 in 1944, and 13.5 in 1945.

It is important to know whether the wild cherries are carriers of the "little cherry" virus. Of the wild cherries listed by Henry (7), *Prunus emarginata* Dougl., is abundant in the Kootenay area and the choke cherry, *Prunus demissa* Nutt., also occurs. In 1943, 2 healthy Lambert trees each received 25 buds of *P. emarginata* from the Willow Point district, where all of the sweet cherry trees have been affected for a number of years. Each one of the buds of *P. emarginata* came from a different tree. Twenty-five definite tissue unions took place, 6 in one tree and 19 in the other. The tree with 6 unions had a good crop of normal fruit in 1944, with the exception of one spur of small fruits, the cause of which was not clear. The tree with 19 unions had a good crop of normal fruit. The experiment was terminated by natural spread of the disease in the orchard in 1945, but it seems probable that at Willow Point, *P. emarginata* is not infected with the "little cherry" virus. No tests have been made with *P. demissa*.

The results obtained in 1944 are shown in Table 1, and substantitate and strengthen the earlier finding that "little cherry" is caused by a virus, and the 1945 results show the extraordinarily rapid natural spread of the disease.

Host Range

The sweet cherry, *Prunus avium* L., has been proven to be affected. The Lambert and Republican varieties seem to be the most severely affected, and the Bing, Black Tartarian, Deacon, and Napoleon varieties

TABLE 1.—TRANSMISSION EXPERIMENTS IN THE KOOTENAY BAY ORCHARD, 1943-1945

No. of trees	Variety	Treatment in 1943	Results in 1944		Results in 1945	
			No. of trees	Condition of fruit	No. of trees	Condition of fruit
16	Lambert	Budded with diseased sweet cherry	12 3 1	Affected Suspicious Normal	16	Affected
4	Republican	Budded with diseased sweet cherry	4	Affected	4	Affected
4	Other	Budded with diseased sweet cherry	4	No crop	2 2	Affected No crop
2	Lambert	Budded with *P. emarginata*	2	Normal	2	Affected
2	Lambert	Root grafted with diseased sweet cherry	2	Normal	2	Affected
19	Lambert	Untreated	10 9	Normal No crop	2 1 16	Normal Suspicious Affected
4	Republican	Untreated	4	Normal	1 3	Normal Affected
9	Other	Untreated	2 7	Normal No crop	1 1 7	Normal Affected No crop

moderately affected. No variety is known to be immune or symptomless. The sour cherry, *Prunus Cerasus* L., shows symptoms similar to those on sweet cherry but was not included in transmission tests. The peach, *Prunus persica* (L.) Batsch, does not appear to become naturally affected. Two peach orchards were for years adjacent to infected cherry orchards but remained free of symptoms of any virus disease. Wild cherries were mentioned above.

NATURAL SPREAD

The manner in which "little cherry" spreads is unknown. The rapidity of spread in an orchard, and from one orchard to another, is so great that the responsibility of one or more insect vectors seems to be indicated. No attempts have been made to transmit the disease by means of insect vectors but both the black cherry aphis (*Myzus cerasi* Fab.) and the apple mealy bug (*Phenacoccus aceris* Sig.) have been commonly observed in affected orchards.

"Little cherry" was first noticed in the Heddle orchard at Willow Point in 1933. In 1934, 3 adjacent orchards were affected. When the first survey was made in 1940, the disease appeared to be general in Nelson and Willow Point and common at Long Beach and Harrop. A few trees were affected near Taghum and Proctor. In one orchard near Proctor 2 trees

appeared to be affected in 1940, and in 1941 all the trees seemed to have "little cherry." In 1945, the disease appeared to be general in Taghum, Nelson, Willow Point, Long Beach, Balfour, Harrop, and Proctor; and patchy at Mirrow Lake, Crawford Bay, Grey Creek, Boswell, Creston, Bonnington, South Slocan, and Robson.

The extraordinarily rapid natural spread of the disease in the experimental orchard at Kootenay Bay has been mentioned above.

DISCUSSION

"Little cherry" does not appear to have been previously recorded in the literature. It resembles the buckskin disease of California in that both diseases cause small fruits. In 1937, D. G. Milbrath (3), who made careful enquiry as to the symptoms in one of the worst affected orchards, was convinced that in any case it was not buckskin. The affected fruits do not develop, just before ripening, the buckskin appearance on the surface of the blossom end (4). The orange to maroon coloration of leaves, exhibited in the early autumn by buckskin affected trees, has never been observed. In trees affected with the Green Valley strain of buckskin, the pedicels of the fruits are abnormally short (5). The pedicels on trees with the Napa Valley strain are normal in length, and the fruits are nearly normal in shape (5). In buckskin, diseased trees vary considerably in vigour, certain individuals remaining in apparently good health for some years, while others make little growth and may have small leaves or dead limbs (4). The buckskin virus causes a leaf casting yellows in peaches (6), and affects the Napoleon cherry variety severely (5). "Little cherry" is, therefore, not the same as buckskin, which is the most nearly similar disease so far described.

SUMMARY

A new disease of sweet cherry is reported from the Kootenay Lake area of British Columbia.

The suggested name "Little cherry," indicates the most striking symptom. The fruits on affected trees are about half the size of those on healthy trees grown under the same conditions, and do not have normal sweetness.

It is a transmissible virus disease.

The spread of "little cherry" within an orchard is very rapid.

No insect vector has yet been found.

The fruits from affected trees are not usually suitable for the fresh-fruit trade, but can be used for processing.

ACKNOWLEDGMENTS

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CHROMOSOMAL STABILITY IN CERTAIN RUST RESISTANT DERIVATIVES FROM A *T. VULGARE* × *T. TIMOPHEEVI* CROSS[1]

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Since new and more virulent strains of the wheat stem rust organism, *Puccinia graminis tritici* E. & H., may appear, it is desirable for the breeder of resistant wheats to have many different sources of resistant germ plasm. He has utilized principally the resistance in the Emmer group of wheats as represented by the use of Iumillo in the production of Thatcher (8) and of Yaroslav Emmer in the development of Hope and H44 (13). Certain of the Kenya wheats, since their discovery, have also shown a high degree of resistance. In addition to these, *T. Timopheevi* zhuk. (2n = 28) has been highly resistant not only to stem rust but to other pathogens (7) and insects to which most of the commonly grown wheats are susceptible. The crosses between *Timopheevi* and the *vulgare* wheats are highly sterile, a difficulty encountered in the use of the Emmer wheats. Both Pridham (18) and Shands (21), however, have isolated *vulgare*-like derivatives from crosses between *T. vulgare* and *T. Timopheevi*. The plants in the present study were obtained from the cross made by Pridham between Steinwedel, a soft, white *vulgare* wheat, and *T. Timopheevi*. The earlier generations were selected for stem and leaf rust resistance under epiphytotic conditions in Australia. Since these lines were obtained from a cross between two species of *Triticum* differing in chromosome number, it seemed desirable to study chromosomal stability in the stem rust resistant derivatives and also to determine if lines combining stability with desirable agronomic characters could be obtained.

LITERATURE

The genome analysis of the genus Triticum has been summarized by Aase (1, 2) and Kostoff (10). Kostoff (10) observed from 8 to 14 bivalents in the F_1 between *T. Timopheevi* and *T. vulgare* which suggested at least a partial homology between the 2 genomes of Timopheevi and 2 of the genomes in the *vulgare* parent. In a cross between the same 2 species, Love (11) observed from 4 to 13 bivalents with an average of 9.5 per pollen mother cell.

Within the 21-chromosome wheats, Sax (20) reported no lagging chromosomes at either Anaphase I or Interphase I in 5 commonly grown varieties. Hollingshead (9), Powers (16), and Love (11) observed various numbers of univalent chromosomes at Metaphase I in a number of *vulgare* wheats, but the percentage reported was small. Elders (7), Hollingshead (9), and Thompson and Robertson (22) reported finding more univalents in most F_1 crosses between varieties than they found in either parent.

In Powers' (16, 17) studies of chromosome behaviour at meiosis in Marquis, Marquillo, and Thatcher, Marquillo was the least stable as measured by the frequency of micronuclei found in microspores. In

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[2] Research Assistant 1939-42. Member of the R.C.A.F. This study was completed under the direction of C. R. Burnham and H. K. Hayes and is being published posthumously. The author was reported missing in action, April 5, 1945.

Marquillo there was also a significant correlation between seed setting and both non-orientation of chromosomes at the first meiotic division and the number of micronuclei in the spore quartets. Myers and Powers (14) observed that Supreme, a *vulgare* wheat, was more unstable than Thatcher, H44, and a sister selection of Thatcher (Double Cross 2305). That such differences were heritable was indicated by their ability to isolate lines in both Marquis and H44 which differed significantly in the frequency of micronuclei.

Love (12) reported that selections from hybrids were being checked cytologically for the presence of microcytes or micronuclei at the quartet stage in order to select the more stable lines for the breeding program.

MATERIAL AND METHODS

Single heads of F_5 and F_6 selections from Pridham's cross of Steinwedel × *T. Timopheevi* were obtained from S. L. Macindoe, formerly with the Glen Innes Experiment Station, Australia. These heads had been selected in Australia from head rows which were resistant or segregating in reaction to a natural epiphytotic of stem and leaf rust. Ten F_5 and 21 F_6 heads were selected as the most desirable lines to continue. Six varieties were used as checks. Of these, Premier, Merit, Thatcher, and Thatcher Backcross were derived from single plant selections, the latter 2 being kindly furnished by Dr. C. H. Goulden and Dr. E. R. Ausemus, respectively. For the other 2, *T. Timopheevi* (C.I. 11802) and Steinwedel (C.I. 4735), bulked seed lots were used.

Thatcher, developed as a co-operative project by the Minnesota Agricultural Experiment Station and the United States Department of Agriculture (8), is a selection from Double Cross (Marquis × Iumillo) × (Marquis × Kanred). Thatcher Backcross, II-31-6, also developed as a co-operative project at the Minnesota Agricultural Experiment Station, is a selection from a backcross of Thatcher × Hope where Thatcher was the recurrent parent. Premier was selected by the North Dakota Agricultural College (15) from a complex cross in which Hope × Florence was crossed with Ceres and a selection from this second cross was hybridized with R.L. 625 (Double Cross × Ceres). Merit was developed from H44 × Ceres by the North Dakota Experiment Station and the United States Department of Agriculture (15).

In order to determine the field rust reaction at St. Paul, Minnesota, an artificial epiphytotic was induced with about 30 of the prevalent physiologic races of *Puccinia graminis tritici*. Rust notes were taken at the time of harvest, the stem rust infections being recorded according to the percentage of infection on the plant and the reaction type as described by Clark, Martin and Stakman (4). The reaction types are as follows:

R—resistant. Pustules very small and round.

MR—moderately resistant. Pustules somewhat larger than in the R class with a tendency toward elongation.

MS—moderately susceptible. Pustules large and elongated but not coalesced.

S—susceptible. Large coalescent pustules with prolific production of urediospores.

Cytological Methods

Chromosome counts were determined from root tips, using Randolph's method (19) with chraf killer and crystal violet stain; or from microsporocytes using acetocarmine smears. The study of chromosome behaviour was based entirely on anther smears. For the smear technique, the heads from first or second tillers were taken, the killer used being a fresh mixture of 3 parts of absolute alcohol and 1 part of glacial acetic acid. After 24 to 36 hr. in the refrigerator the killing fluid was replaced by 70% alcohol and again stored in a refrigerator at about 36° F. Material prepared and stored in this manner was found suitable for cytological examination even after 15 mo. Plants sampled for cytological studies were tagged to facilitate the taking of detailed plant notes.

The following anomalies and characters were studied:

Metaphase I.	Univalent formation non-oriented bivalents precocious bivalents
Anaphase I.	Lagging univalents precocious univalents
	Lagging bivalents precocious bivalents
Interphase I.	Micronuclei and microcytes
Metaphase II.	Non-oriented chromosomes and microcytes
Anaphase II.	Precocious chromosomes lagging chromosomes microcytes
Quartets.	Micronuclei microcytes
Pollen.	Abnormal pollen grains
Seed set.	Seed set in the lateral florets seed set in all florets of the spike

A few of the above terms may need explanation. Non-oriented chromosomes were those located at some distance off the equatorial plate. Chromosomes which were closer to the poles than the main body of chromosomes were classed as precocious.

An attempt was made to study all the above stages in each plant. All cells on a slide that could be positively described were recorded, but no counts were made of the number of cells that could not be observed clearly. Only paired cells were considered in Division II.

To determine pollen sterility, the entire contents of an anther were squeezed into a drop-potassium iodide solution on a slide and a cover slip added. All grains of normal size and more than $\frac{1}{2}$-filled with starch were

considered normal. Usually the normal-sized grains were completely filled. All the grains, taken in successive strips across the slip, were recorded, usually between 200 and 700 pollen grains from each anther. Two to 3 such counts were made, each with a single anther from a different floret. Results based on 20 to 30 random fields agree very well with those determined from the entire slide.

Seed set was determined for both the lateral and central florets, omitting the lower 2 spikelets and the terminal 3 or 4. The total number of spikelets per plant on which seed set was determined ranged from 16 to 40, depending on the number of spikes available.

EXPERIMENTAL RESULTS

Of the 31 different selected Steinwedel × Timopheevi lines, each represented by a single head row sown in the spring of 1940, 10 were of the winter growth habit and did not head, 6 were very late in heading, 9 were of the spring type and headed relatively early, while the remaining 6 were segregating for early and late heading as well as winter growth habit. It is interesting to find such a large number of derivative lines with winter habit from a cross between 2 different spring wheat species.

The tests for rust reaction at St. Paul showed that 11 of the 21 lines which headed contained plants resistant to stem and leaf rust in the field. At the end of 2 further generations of limited testing, 2 lines were apparently homozygous for leaf rust resistance but were still segregating for stem rust reaction.

Cytogenetic Studies of Derived Lines with Spring Habit

As to chromosome number, 55 plants in 19 lines were examined. All but one had approximately the chromosome number of the *vulgare* parent, the one exception being a plant with 28 chromosomes. One plant of the Steinwedel variety had 20 bivalents and 1 univalent.

In order to determine the significance of differences in chromosome behaviour at meiosis, the 1942 data on the percentages of normal cells at Metaphase I, Anaphase I, and of normal quartets and pollen were used. Since 2 or more anthers were studied per plant, the actual differences within plants were used in calculating a generalized error. No cases based on fewer than 24 cells from a single anther were included in this analysis. The actual percentages were used, since calculations using transformed percentages as suggested by Bliss (3) and Clark and Leonard (5) gave similar values. The values are as follows:

Stage	No. of plants studied	No. of anthers per plant	Level of significance
Metaphase I	20	2	10.3
Anaphase I	13	2	9.5
Quartets	13	2	6.1
Pollen	29	3	11.1

* Difference necessary for significance at the 5 % point.

TABLE 1.—CORRELATION COEFFICIENTS BETWEEN PERCENTAGES OF CHROMOSOMAL ANOMALIES IN THE VARIOUS STAGES OF MEIOSIS, ABORTED POLLEN, AND SEED STERILITY IN 8 DERIVED (STEINWEDEL × *Timopheevi*) LINES WITH SPRING HABIT. (BASED ON PLANTS IN 1941 AND PROGENY IN 1942 GROWN IN THE FIELD AT UNIVERSITY FARM, ST. PAUL)

	Anaphase I					Inter-phase	Meta-phase II	Anaphase II			Quartet	Aborted pollen	Seed sterility	
	Univalent			Bivalent	Total			Precocious	Lagging	Total			Lateral florets	All florets
	Lagging	Precocious	Total	Total										
Metaphase I, univalents	.802*	—	.831*	.418	.833*	.885*	.778*	—	.525*	.829*	.895*	.788*	.437†	.052
Metaphase I, precocious bivalents	—	—	.224	—	.017	.722*	.100	—	—	.302	.404	—	—	—
Metaphase I, non-oriented bivalents	.009	.324	—	—	.034	−.098	.267	−.325	.056	−.267	−.083	—	—	—
Metaphase I, total abnormalities	—	—	—	—	.814*	.893*	.702*	—	—	.820*	.866*	.630*	.320	.223
Anaphase I, bivalent lagging	—	—	—	—	—	.622*	.576	—	—	—	—	—	—	—
Anaphase II, bivalent total	—	—	—	—	—	.345	.431†	—	—	.658	.547	—	—	—
Anaphase II, total univalents	—	—	—	—	—	.754*	.755*	—	—	.954*	.821*	—	—	—
Interphase	—	—	—	—	—	—	.783*	—	—	.852*	.686*	.667*	.766*	.374
Metaphase II	—	—	—	—	—	—	—	—	—	.840*	.864*	.501†	.388	.217
Anaphase II	—	—	—	—	—	—	—	—	—	—	.961*	.758†	.528†	.356
Quartet micronuclei	—	—	—	—	—	—	—	—	—	—	—	.788*	.562	.399
Percentage aborted pollen	—	—	—	—	—	—	—	—	—	—	—	—	.576*	.165

* Highly significant, exceeding the 1% point.
† Significant, exceeding the 5% point.

Since the data (percentages of normal cells) were taken from many plants, the differences recorded as necessary for significance at the 5% point for each stage may be applied, with caution, to all plants studied in 1942. Differences of about 10% were necessary for significance at the 5% point for metaphase I and anaphase I, 6% for quartets, and 11% for pollen.

The data from the cytological study of plants of the 8 derived (Steinwedel × Timopheevi) lines of spring growth habit grown in 1941 and progeny grown in 1942 were used in calculating correlation coefficients between the percentage frequencies of abnormalities at different stages and are shown in Table 1. The data on the kinds and frequencies of abnormalities on which these correlations are based are presented in Tables 2, 3 and 4. The correlations are considered along with the data since they aid in considering the cytological behaviour of the chromosomes. The frequency of univalents at metaphase I was significantly correlated with the frequencies of abnormalities at all the later stages except lagging bivalents at anaphase I and sterility in the entire head. The significant correlations were with anaphase I univalents, and the abnormalities at interphase, metaphase II, anaphase II, and in spore quartets; as well as pollen abortion and sterility in the lateral florets. All values were high and highly significant ($P = < .01$), except the latter which was low but significant ($P = < .05$). The frequencies of precocious and non-oriented bivalents at metaphase I showed low and non-significant correlations with the frequencies of anomalies at later stages except for a highly significant correlation between precocious bivalents and micronuclei at interphase.

The detailed data on these metaphase I abnormalities are in Table 2. The frequencies of cells showing univalents range from 4.8 to 53.8% for different lines. There was also wide variation between plants of the same line, e.g. 3 plants in line 1013 had univalents in 50, 53.8 and 7%, respectively of their cells. Since correlations with abnormalities at later stages are high and highly significant, it is possible that the univalent chromosomes at metaphase I behave abnormally at later divisions.

Some additional evidence as to the behaviour of univalent chromosomes was furnished by the 41 chromosome plant of Steinwedel which showed 20 pairs and 1 univalent. Univalents were found in only 76% of the cells at metaphase I, and micronuclei in only 25% of the diads at interphase, later stages not being available. Powers (16) observed in a 41-chromosome plant the extrusion of chromatin material from the nucleus at early prophase in 9.4% of the microsporocytes. If this represents the univalent chromosome, and if it is a common behaviour in such plants, it would account at least in part for the fact that a univalent was observed in fewer than 100% of the sporocytes. Since only 25% of the diads showed micronuclei, many of the univalents must have been included in the daughter nuclei at the first division. These might be expected to behave normally in the following stages. Any that divided at the first division on the metaphase plate would be expected to behave abnormally in second division stages. The high correlations suggest that many univalents divided at the first division.

Only a few plants in the 4 varieties; Premier, Merit, Thatcher, and Timopheevi were studied; but the frequency of metaphase I univalents was

relatively low, one plant of Thatcher being highest with 11.7%. Two of the derived lines, 996 and 1016, probably did not differ significantly from the varieties.

Of the other abnormalities at metaphase I, non-oriented bivalents were relatively frequent in certain plants but not more frequent in the derived lines than in the parents (Table 2). Although they might result in micronuclei if not on the spindle, the lack of correlation with behaviour at later stages seems to indicate relatively normal subsequent behaviour. The precociously divided bivalents at metaphase I were infrequent (Table 2) and showed correlation only with the formation of micronuclei at interphase. They might be expected to merely arrive at the poles in advance of the main groups, and behave normally at the later stages.

TABLE 2.—PERCENTAGES OF CELLS AT METAPHASE I SHOWING UNIVALENT, NON-ORIENTED BIVALENT AND PRECOCIOUS BIVALENT CHROMOSOMES IN 4 VARIETIES AND 8 DERIVED SPRING LINES OF (STEINWEDEL × *T. Timopheevi*) GROWN IN IN 1941 AT UNIVERSITY FARM, ST. PAUL

Variety or line No.	Plant No.	Total cells	Per cent of metaphase I cells with the following anomalies			
			Univalents	Non-oriented bivalents	Precocious bivalents	Total abnormal cells
Premier	1–1	553	6.1	12.6	1.3	19.9
Premier	–3	373	7.2	2.9	0.0	8.8
Merit	149–7	208	1.4	23.6	0.0	26.4
Thatcher	150–3	196	11.7	2.0	2.6	16.8
T. Timopheevi	109–10	713	2.1	3.6	0.7	6.4
T. Timopheevi	109–20	156	1.9	1.3	1.3	4.5
996	16–1	261	8.4	10.0	0.0	18.4
1010	20–2	113	12.4	4.4	1.8	16.8
1011	23–1	173	23.7	1.7	11.2	30.0
1011	23–2	73	12.3	13.7	1.4	24.7
1013	31–4	98	50.0	2.0	6.1	59.2
1013	31–5	427	7.0	1.6	0.9	9.1
1013	33–4	264	53.8	4.6	6.1	63.3
1014	35–1	308	10.1	7.1	1.6	17.8
1016	41–3	234	17.5	3.8	2.1	17.5
1016	42–3	83	4.8	3.6	0.0	8.4
1016	46–2	—	—	—	—	—
1017	52–2	105	16.2	14.3	1.9	28.6
1017	56–1	358	18.4	9.8	1.7	28.2
1018	64–1	449	32.3	1.8	1.3	34.5

The abnormally oriented univalents and bivalents at anaphase I show significant correlations with abnormalities observed at interphase, metaphase II, anaphase II, and quartets; no values being available for pollen abortion and floret sterility.

The detailed data on the kinds and frequencies of abnormalities at anaphase I are in Table 3. With two exceptions, the number of abnormally oriented univalents and bivalents at this stage is relatively low, the totals for these 2 plants being 46.8 and 26.6%, respectively while those for the other 12 plants representing the derived *Timopheevi* × *Steinwedel* lines were 6.2% or less. The highest value for the *vulgare* varieties was 2.7%.

TABLE 3.—PERCENTAGES OF CELLS SHOWING VARIOUS ANOMALIES AT ANAPHASE I IN 4 VARIETIES AND 8 DERIVED SPRING LINES OF STEINWEDEL × *T. Timopheevi*)

Variety or line No.	Plant No.	Total cells	Percentage anaphase I cells with the following anomalies				Total % with anomalies	Interphase diads	
			Univalent lagging	Univalent precocious	Bivalent lagging	Bivalent precocious		Total No.	% With micro-nuclei
Premier	1–1	531	1.0	1.7	0.0	0.0	2.7	248	2.0
Premier	1–3	303	0.3	0.3	0.3	0.0	0.7	5	40.0
Merit	149–7	85	0.0	0.0	1.2	0.0	1.2	—	—
Thatcher	150–3	320	0.6	1.9	0.9	0.0	2.2	95	6.3
T. Timopheevi	109–10	258	0.4	0.8	0.4	0.4	1.9	357	2.5
T. Timopheevi	109–20	403	0.2	0.5	0.2	0.5	1.5	83	0.0
996	16–1	28	0.0	0.0	0.0	0.0	0.0	—	—
1010	20–2	43	0.0	0.0	0.4	0.0	0.4	46	2.2
1011	23–1	227	0.4	3.6	0.9	0.4	5.3	39	33.3
1011	23–2	61	0.0	4.9	0.0	0.0	4.9	14	92.9
1013	31–4	—	—	—	—	—	—	—	—
1013	31–5	240	3.3	3.1	0.0	0.8	6.2	117	3.4
1013	33–4	47	34.0	2.1	10.6	0.0	46.8	151	66.2
1014	35–1	97	1.0	1.0	2.1	0.0	3.1	4	0.0
1016	41–3	251	0.4	0.8	0.0	0.0	1.2	54	11.1
1016	42–3	69	1.4	0.0	0.0	0.0	1.4	50	2.0
1016	46–2	158	1.9	0.0	0.6	0.0	2.5	169	6.5
1017	52–2	31	0.0	0.0	0.0	0.0	0.0	78	25.6
1017	56–1	189	1.6	0.0	1.1	0.5	2.1	5	0.0
1018	64–1	384	22.1	0.0	4.4	0.0	26.6	167	38.3

The frequency of micronuclei at interphase is correlated with subsequent abnormal behaviour at metaphase II, anaphase II, spore quartets, pollen abortion, and sterility of the lateral florets; all values being high and highly significant. The data on micronuclei in the diads at interphase are presented in the last 2 columns of Table 3. Wide variation within lines as well as between lines was observed, the range for plants in which at least 40 diads were observed being from 2.0 to 66.2%. The *vulgare* varieties showed low percentages except for one plant of Premier with 40%, but this is again based on only 5 diads which is too small a population.

The data on the percentages of abnormal diads at meiosis II, spore quartets, pollen, and also lateral floret sterility are shown in Table 4. A wide range of variation in frequency was observed from low to extremely high values except for sterility in lateral florets which was relatively high in all plants. As to the correlations (Table 1), the frequency of non-oriented chromosomes at metaphase II showed highly significant correlations ($P = < .01$) with abnormal behaviour at anaphase II, and with micronuclei in spore quartets; a significant correlation ($P = < .05$) with pollen abortion, but none with floret sterility. Abnormalities at anaphase II were correlated ($P = < .01$) with abnormal spore quartets and correlated ($P = < .05$) with pollen abortion and lateral floret sterility.

The frequency of quartet micronuclei was correlated with pollen abortion and just below $P = .05$ with lateral floret sterility. The percentage of normal pollen was correlated with fertility of the lateral florets ($r = .576$).

The frequencies of these abnormalities in the few plants of the *vulgare* varieties are relatively low. One plant of Premier had high percentages of abnormal metaphase II diads, of abnormal pollen, and of sterile lateral florets, although the abnormalities at the other stages, including metaphase I, were relatively low in frequency.

Cytological Study of Derived Lines with Winter Habit

Twelve plants representing 4 different lines with winter growth habit were grown in the greenhouse in 1941 and studied cytologically. One plant of the check variety Thatcher had 25% of its cells abnormal at metaphase I but was relatively normal in the later stages.

The plants of the *Timopheevi* derivatives with winter habit showed at most stages a greater variation and higher percentages of abnormalities than the lines with spring habit. At metaphase I, the frequency of abnormal cells varied from 21 to 68%. At the quartet stage the variation in frequency of abnormal sporads was from 5 to 36%. Only 1 plant of line 986 approached the stability of Thatcher.

Cytological Study of Progeny of Derived Lines with Spring Habit

To determine the breeding behaviour as concerns chromosomal stability, 6 plants of spring habit differing in stability were selected as parents for progenies to be studied cytologically. The stability of the parent plants, representing 4 different lines, and their progeny may be judged by the frequencies of cells with univalents at metaphase I:

1941		% Of cells with univalents at metaphase I	
Line	Plant number	In the parent plant	In progeny, data on different plants, 1942
996	16–1	8.4	6.6, 5.6, 5.6
1011	23–1	23.7	18.4, 12.7*
1013	31–4	50.0	20.5*, 14.3*, 20.0*, 27.6*
1013	31–5	7.0	16.3, 12.7, 16.0
1016	41–3	17.5	13.6 8.1
1016	46–2	—	4.6, 5.7

* Progeny which differed significantly from their parent plants.

The progeny of 16–1, 41–3, and probably 46–2 (as judged by all stages) were very similar to their respective parents. All the progency of 31–4 had a significantly lower percentage of cells with univalents. The 3 progeny of 31–5 had uniformly higher percentages. Data on the frequencies of other abnormalities are in Table 5, together with those of the parents for comparison. In several cases, data on the parent were not available so that comparisons could not be made. With few exceptions, the offspring showed very similar frequencies of abnormalities at corresponding stages. One exception was at interphase; the parent 23–1 having 33.3% while the 2 offspring had 6.9 and 4.3% of diads with micronuclei. These same progeny, however, were very similar to the parent in behaviour at all the other stages. As a statistical measure of the similarities, correlation coefficients were calculated. That between the frequencies of chromosomal abnormalities in the parent and the average frequency of the same

anomaly in the offspring was r = 0.59 which is highly significant. If only those types of abnormalities which were found to be correlated within the same plant (i.e. univalents at metaphase I and anaphase I; anomalies at interphase, metaphase II, anaphase II, in spore quartets; and pollen abortion as shown in Table 1) are used, r = 0.73, a high value which is highly significant. These correlations, and the fact that lines differing in stability were established indicate that the differences in chromosomal stability were inherited.

TABLE 4.—PERCENTAGE FREQUENCIES OF ABNORMALITIES IN SECOND DIVISION STAGES OF MEIOSIS, TOGETHER WITH DATA ON POLLEN AND OVULE ABORTION IN 4 VARIETIES AND IN 8 DERIVED SPRING LINES FROM (STEINWEDEL × *Timopheevi*)

Variety or line No.	1941 plant No.	Metaphase 11 diads		Anaphase 11 diads		Spore quartets		Pollen	Seed set (laterals)
		Total No.	% Abnormal	Total No.	% Abnormal	Total No.	% With micro-nuclei	% Abnormal	% Sterile florets
Premier	1–1	131	1.5	4	0.0	—	—	—	38
Premier	1–3	15	13.3	337	0.9	341	2.6	93.3	56
Merit	149–7	—	—	—	—	217	5.5	0.5	—
Thatcher	150–3	44	11.4	104	2.9	209	1.4	5.2	—
Timopheevi	109–10	279	0.4	154	1.3	—	—	1.5	—
Timopheevi	109–20	—	—	—	—	873	0.0	0.8	—
996	16–1	—	—	—	—	—	—	1.6	33
1010	20–2	—	—	—	—	358	11.2	35.6	27
1011	23–1	142	14.1	110	5.5	423	8.3	1.9	36
1011	23–2	84	13.1	139	6.5	105	13.3	—	—
1013	31–4	114	23.7	138	23.9	641	33.5	37.4	52
1013	31–5	—	—	—	—	657	6.7	86.9	26
1013	33–4	105	41.0	198	58.1	60	60.0	61.1	—
1014	35–1	44	6.8	101	6.9	718	6.4	10.4	67
1016	41–3	110	17.3	189	9.0	333	5.7	15.5	28
1016	42–3	42	0.0	74	0.0	29	0.0	6.8	21
1016	46–2	268	4.9	189	2.7	196	1.0	11.9	30
1017	52–2	189	20.6	222	7.7	25	8.0	2.2	30
1017	54–1	55	9.1	140	6.4	565	6.0	5.9	21
1017	56–1	—	—	—	—	—	—	2.9	—
1018	64–1	103	46.6	164	36.6	60	31.7	1.8	—

Notes were taken on a number of agronomic characters of all plants that were studied cytologically but only the description of 4 progenies with comparatively high chromosomal stability as shown by the percentage of univalents in metaphase I cells are summarized in Table 6. It is observed from this table that it is possible to combine resistance to leaf and stem rust in a line with *vulgare* characteristics possessing as great chromosomal stability as Premier and Thatcher.

CYTOLOGICAL STUDY OF HYBRIDS

One of the lines with winter habit (984) was crossed with both the Premier and Merit varieties. Cytological study of the F_1 plants indicated they were much more unstable than either parent (Table 5). Pollen diameters were also more variable in the F_1 plants than in the varieties. This indicates that when these derived lines are used in crosses, selection for chromosomal stability in succeeding generations may be necessary.

TABLE 5.—CYTOLOGICAL BEHAVIOUR IN PROGENY OF SELECTED PLANTS DIFFERING IN CHROMOSOMAL STABILITY IN 3 VARIETIES AND IN CERTAIN F_1 AND F_2 OF CROSSES BETWEEN (STEINWEDEL × *Timopheevi*) DERIVATIVES AND THE HARD RED SPRING WHEATS, *Premier* AND *Merit*, GROWN IN FIELD IN 1942 AT UNIVERSITY FARM, ST. PAUL

Variety or line No.	1941 plant No. (parent)	1942 plant No.	Anaphase I		Interphase diads		Metaphase II diads		Anaphase II diads		Quartet		Pollen
			Total No.	Abnormal %	Total No.	Abnormal %	Total No.	Abnormal %	Total No.	Abnormal %	Total No.	Abnormal %	Abnormal %
Premier	—	7–2	—	—	—	—	—	—	207	2.41	740	2.4	—
Steinwedel	—	6–2*	258	25.6	12	25.0	—	—	—	—	—	—	—
Steinwedel	—	6–4	300	5.0	84	6.0	—	—	—	—	—	—	2.1
Premier	—	7–2	185	6.0	298	1.0	82	1.2	9	0.0	—	—	—
Thatcher	—	16–1	392	2.6	300	2.0	105	3.8	65	3.1	558	2.0	0.8
Thatcher	—	16–2	603	1.0	264	.38	116	0.0	—	—	585	0.5	—
996	(16–1)	—	—	0.0	—	—	—	—	—	—	—	—	1.6
	—	706–1	—	—	—	—	—	—	—	—	—	—	1.0
		–3	215	3.7	250	4.0	124	1.6	206	2.4	109	1.8	0.7
		–4	56	3.6	141	3.6	25	12.0	100	4.0	341	10.0	3.6
1011	(23–1)	—	—	5.3	—	33.3	—	14.1	—	5.5	—	8.3	1.9
		708–1	306	5.2	233	6.9	53	15.1	218	2.3	426	4.2	0.9
		–5	143	6.3	93	4.3	—	—	—	—	453	5.5	3.0
	—	–6	—	—	—	—	—	—	—	—	—	—	—
1013	(31–4)	—	—	—	—	—	—	23.7	—	23.9	—	33.5	37.4
		31–4–1	169	31.4	42	11.9	—	—	—	—	560	22.0	—
		–2	173	9.8	71	14.1	30	23.3	135	12.6	809	8.4	—
		–3	19	36.8	44	32.2	52	15.4	217	28.1	723	27.8	—
		–5	111	27.0	61	19.7	—	—	—	—	—	—	—
1013	(31–5)	—	—	6.2	—	3.4	—	—	—	—	—	6.7	86.9
		31–5–1	122	2.5	134	12.7	90	10.0	163	11.7	560	6.6	—
		–2	—	—	—	—	—	—	—	—	355	15.2	—
		–6	141	14.2	285	9.1	53	15.1	165	13.9	388	11.1	9.6
1016	(41–3)	—	—	1.2	—	11.1	—	17.3	—	9.0	—	5.7	15.5
		734–6	439	3.2	215	7.4	26	15.4	132	14.4	705	8.6	3.2
		–8	137	7.3	274	13.5	151	6.0	142	4.2	—	—	3.0
1016	(46–2)	—	—	2.5	—	6.5	—	4.9	—	2.7	—	1.0	11.9
	—	758–1	304	3.0	256	8.2	55	7.3	142	2.8	550	2.4	1.7
	—	–2	—	—	138	13.0	128	7.0	227	0.4	369	2.4	2.2
Premier × 984 F_1	—	C 2–3	9	0.0	53	60.4	89	39.3	204	32.8	276	17.0	14.5
Merit × 984 F_1	—	C13–1	—	—	23	52.2	31	38.7	95	27.4	291	27.8	9.9
986 × Premier F_2	—	C50–5	306	5.2	31	0.0	—	—	—	—	—	—	—
986 × Premier F_2	—	C61–6	153	7.2	6	0.0	—	—	—	—	—	—	2.5

*2n-1.

TABLE 6.—PERCENTAGE OF CYTOLOGICAL ABNORMALITIES AND AGRONOMIC DATA FOR *Thatcher*, *Premier* AND 4 PROGENY ROWS OF *Timopheevi* DERIVATIVES GROWN IN THE RUST NURSERY IN 1942 AT UNIVERSITY FARM, ST. PAUL

Variety or *Timopheevi* derivative	1942 row No.	Date of heading	Clume		Lodging %	Height of plants	Stem rust reaction	Leaf rust infection	Univalents in meta-phase I %	Normal pollen %	Abnormal quartets %	Seed set lateral florets %
			Cover	Colour								
Thatcher	—	June 26	Glabrous	White	—	31	R	60	6.8	99	1.3	81
Premier	—	June 25	Glabrous	White	25	41	R	Trace	9.9	—	—	—
996	706	June 29	Glabrous	White	50	36	R	Trace	5.9	98	5.9	81
1011	708	June 28	Pubescent	White	12	36	R	Trace	15.6	98	4.9	87
1016	734	June 24	Glabrous	White	12	37	R	20	10.9	97	8.6	88
1016	758	June 26	Glabours	White	25	35	Seg.	5	5.2	98	2.4	92

DISCUSSION

From the standpoint of the plant breeder, a usable criterion of chromosomal stability would be useful. Seed set appears to be of little use. Estimates of pollen sterility would be the easiest to use and would serve to identify many of the highly abnormal plants. However, certain cases were observed in this study in which high frequencies of abnormalities at meiosis were accompanied by low pollen abortion. This may be due to the fact that in a polyploid such as *vulgare* wheat chromosomal deficiency does not necessarily result in spore abortion.

The frequency of quartets or microspores with micronuclei might be applied as a final check on the most desirable lines. The frequency of univalents at metaphase I, micronuclei at interphase, and abnormalities at metaphase II would also be useful criteria in case these other stages were found while searching for the quartet stage.

SUMMARY

Ten F_5 and 21 F_6 selections made by Pridham from the cross of Steinwedel, a soft, white *vulgare* wheat, and *T. Timopheevi* (2n = 28) were used in a study of chromosomal stability.

As to chromosome number, 54 plants in 19 derived lines had approximately the normal *vulgare* number, and one had 28 chromosomes. One plant of Steinwedel had 20 bivalents and 1 univalent.

The varieties used as checks showed 1.4 to 11.7% of cells wiht univalents at metaphase I, but were relatively normal in later stages.

In 15 plants representing 8 Steinwedel × *Timopheevi* lines with spring growth habit, univalent chromosomes were observed in 4.8 to 53.8% of the cells.

Correlations were highly significant between the percentage of metaphase I cells with univalents and the following: total abnormalities at anaphase I, metaphase II, and anaphase II; micronuclei at interphase and in spore quartets; and aborted pollen. All showed correlations high enough to be used in selection as criteria of chromosomal stability.

Progeny of 6 plants differing in chromosomal stability were studied cytologically. Differences in stability are inherited as indicated by the fact that parent-offspring correlations for abnormalities at corresponding stages were highly significant and that lines differing in stability were established.

Derivative lines with winter habit were chromosomally less stable on the average than those with spring habit.

Two F_1 hybrids between a Steinwedel × *Timopheevi* derivative line with winter habit and Premier and Merit were much more unstable than either parent.

It was possible to select lines from Pridham's material which combined high chromosome stability with *vulgare* characteristics and resistance to stem and leaf rust.

The percentage of pollen abortion may be useful to the plant breeder in eliminating many of the highly unstable lines. The frequency of micronuclei in spore quartets or microspores might be used later in the selection program to check the most desirable lines.

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THE ROLE OF CALCIUM, PHOSPHORUS, SULPHUR AND SUPERPHOSPHATE FOR TOBACCO[1]

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Superphosphate fertilizer supplies 3 major essential elements of plant food, viz., phosphorus, calcium and sulphur. In tobacco investigations involving different quantities of superphosphate, a knowledge of the role of each of these elements is of fundamental importance. Accordingly, some pertinent functions of phosphorus, calcium and sulphur in respect to plant growth, soil reaction, yield and quality of leaf produced, are individually reviewed and discussed in this preliminary paper. Subsequent papers will deal with the effect of superphosphate on the maturity and nitrogen metabolism of tobacco.

Most of the calcium applied annually to Canadian tobacco soils is carried by superphosphate. The cost of this calcium is charged to the phosphorus content of the fertilizer. Sulphur is supplied more freely to tobacco soils than any other nutrient in such fertilizer materials as superphosphate, sulphate of potash and sulphate of ammonia. Recent investigations (3, 32, 47, 51, 75, 87) indicate that calcium, magnesium, boron, sulphur, manganese and iron should be provided for *in reasonably proper proportions* as well as NPK in fertilizer mixtures. In Connecticut (4), where organic fertilizers are available and extensively used by tobacco growers, these requirements appear to have been met successfully. However, where farmers depend on mineral fertilizer salts, it has seldom been possible to provide the *proper* quantity of these essential elements in commercial NPK mixtures. The commercial production and use of such salts as the nitrate, carbonate or silicate of potash in commercial NPK mixtures would substantially reduce the sulphate content and result in a more rational proportion of sulphur in tobacco fertilizers.

The intensive studies by Ward (87) and McEvoy (47) of the Tobacco Division have contributed to the knowledge of the fertilizer requirements of Canadian tobacco. The fertilizer problem seems to be of sufficient importance, therefore, to justify consideration from many approaches. In the present treatise, considerable attention is given to the common carriers of calcium and sulphur besides superphosphate, to ionic-equivalents as a method of expressing plant nutrient data, and to the relation of soil pH to the absorption of H_2PO_4 ions by plants.

SUPERPHOSPHATE

Commercial superphosphate, 16% P_2O_5, is a mixture of mono-calcium phosphate, $Ca(H_2PO_4)_2 \cdot H_2O$, about 30% and anhydrous calcium sulphate about 50%. Its composition varies according to the source of rock used, even to different locations of the same deposit, the age of the prepared product and the method of preparation. The minor constituents of superphosphate, which may vary more than the major constituents, include silica about 5%, fluorine about 1.5%, Fe_2O_3 from 0.6 to 2.1%, Al_2O_3 from

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0.5 to 1.3%, Na_2O about 0.35%. Values of 0.16% K_2O, 0.53% organic matter, 4.87% moisture and 12.10% combined water have also been reported. The composition given in Table 1, an average of 8 analyses (27, 39), is therefore arbitrary for use in this paper.

Recently in Eastern Canada, the 16% grade has been replaced by a 20% grade of superphosphate which contains about 16% P_2O_5 as monocalcium phosphate and in addition about 5% P_2O_5 as dicalcic phosphate. The percentage of SO_3 is slightly less, but otherwise the "20% grade" does not appear to alter materially the composition shown in Table 1.

TABLE 1.—AVERAGE COMPOSITION OF COMMERCIAL SUPERPHOSPHATE (16% Grade)

		%	
Sulphuric acid	(SO_3)	29	
Lime	(CaO)	28	
Phosphoric acid total	(P_2O_5)	20	(16 to 18% available)
Free and combined water		14	
Oxides of minor elements, etc.		9	
Total		100	

CALCIUM

ROLE IN PLANTS

Calcium is indispensable to the growth and development of all higher plants (8, 50, 54, 66, 69). The failure of normal mitosis of plant roots (74) and leaves (8, 51) when this nutrient element is withdrawn, and its presence in the cytoplasm of cabbage leaves (24), indicate that calcium may be an essential element of protoplasm. It appears to function in diastase activity and to be necessary for carbohydrate translocation (54). Nightingale (60) found that nitrates were not absorbed or assimilated without calcium. Consequently, it also may be an important initial factor in nitrogen metabolism.

Compounds of calcium occurring in plants include salts, chiefly of organic acids and possibly some inorganic acids, also 2 known nitrogen-free lipids. Calcium pectate serves as a structural component of the middle lamella of the cell wall and functions in the retention of ions of the plant cells. Parker and Truog (62) concluded that calcium neutralizes the organic products of protein metabolism, particularly oxalic, acetic, succinic and formic acids, accumulations of which would otherwise interfere with further protein metabolism. The two lipids are calcium phosphatidate (calcium diglyceride of phosphoric acid) discovered in cabbage leaf cytoplasm by Channon and Chibnall (24) and phytin, a calcium-magnesium salt of inositol phosphoric acid (34). A large proportion of the calcium is water-soluble and easily extracted from the dried tissue (31, 54).

Dried cured tobacco may contain from 2 to 8% of CaO (8). An apparent excess of magnesium, potassium and possibly boron in plants, may result from a calcium deficiency and vice versa (2, 55, 66, 69). Without calcium, magnesium may be toxic in plants (50). Investigations (66, 69) suggest that calcium may exert a balancing effect toward magnesium and potassium and other ions within the plant. Calcium deficiency in tobacco soils is not common because of the usual heavy fertilization with materials

containing it, however, calcium deficient soils may produce stunted plants and cause leaves in the terminal bud to turn brown, curl up and die (8, 50). Swanback *et al.* (78) considered that calcium and nitrogen were very important limiting factors in tobacco plant growth.

Nutrient Relations of Cigar Tobacco

The importance of calcium in tobacco nutrition is amplified in Table 2. At Ottawa, an average of 123 lb. CaO was absorbed by an acre of cigar tobacco which shows that the plant capacity to absorb calcium is high which was also found by Ward (87) for flue-cured tobacco. An amount equal to 60% of the calcium content of superphosphate applied to soil annually in a basal ration was removed by a cigar tobacco crop. The calcium content of dried cigar leaf averaged about 6% CaO (87). Garner (31) concluded that lime in general does not affect the fire-holding capacity but is an essential factor in producing a good cigar ash. Ames and Boltz (2) concluded that a high lime content causes objectionable flaking of the ash. Both viewpoints have been confirmed by Anderson and co-workers (55) with cigar wrapper and binder tobacco; an adequate calcium content was found to improve taste, aroma, burn and general quality of cured and fermented leaf.

Table 2.—Quantities of phosphorus, sulphur and calcium, compared with potassium, applied annually to soil in basal ration[1] and removed by cigar tobacco crop

Expressed as oxides	P_2O_5 lb./ac.	SO_3 lb./ac.	CaO lb./ac.	K_2O lb./ac.
Applied as superphosphate 750	120	217	210	—
Sulphate of potash 360	—	147	—	173
Sulphate of ammonia 160	—	91	—	—
Cottonseed meal 1420	40	—	—	27
Total applied to soil	160	455	210	200
Removed by crop (3 yr. av.)[2]	26.4	55	123	235.8
Unused excess applied to soil	133.6	400	87	—

[1] Basal ration for cigar tobacco consisted of 160 lb. N, 160 lb. P_2O_5 and 200 lb. K_2O per acre.
[2] From tobacco investigations by Ward (87).

Soil Reaction and Black Rootrot

The majority of Canadian tobacco soils are predominantly acid in reaction, although some tobacco is grown on neutral or slightly alkaline soils (52, 16A). The calcium in these soils is replenished annually, chiefly by superphosphate contained in commercial fertilizer mixtures and also sometimes to a much less extent by dolomitic limestone used as filler material to furnish magnesium. Indiscriminate liming of tobacco soils is not recommended. Excessive liming produces a soil condition favourable to black rootrot activity (44, 55). Liming experiments carried on in the United States and Canada before 1929 involved application of rates from 1000 to 4000 lb. CaO per acre regardless of soil pH. These experiments were reviewed by Morgan, Anderson and Dorsey (55). They pointed out that in nearly every case both the tobacco yield and quality were reduced;

enough lime was applied to increase the soil pH beyond 5.8; black rootrot, insofar as it was observed, was particularly severe in soils with pH's above 6.4.

Morgan *et al.* (55) found that black rootrot damage to susceptible varieties of cigar tobacco was prevalent at soil pH's above 5.6 and that the most satisfactory crops were grown at a soil reaction range of pH 5.0 to 5.6. Strongly acid soils with pH's less than 4.8 produced tobacco of low yield and quality with a high content of manganese which was toxic to the crop and resulted from a high concentration of soluble manganese in these soils. The addition of lime to very acid soils to raise the pH to 5.6 improved the yield, burning quality, taste and aroma of the tobacco. On soils testing 5.0 to 4.6 pH, 1000 lb. application of agricultural limestone per acre was recommended and on soils below 4.6 pH, 2000 lb. per acre, except on excessively sandy soils when these rates should be somewhat smaller. Soils testing above pH 6.0, infested with black rootrot fungi, were considered unsafe for susceptible varieties of tobacco (6, 55). Regarding soil reaction tests, Swanback and Morgan (10, 77) found that soil pH may decrease by as much as 1.0 unit during a dry hot summer. They observed that a mean pH value occurred in April and October and concluded that these months were optimum times for testing soil reactions. These pH changes partially explain the recovery of tobacco from black rootrot in midsummer when the soil becomes warmed up (59).

Gypsum is an important source of calcium for cigar tobacco (7). It is of interest that about 50% of the calcium sulphate contained in superphosphate is water soluble (30). Compared to lime, calcium sulphate may have little effect on soil reaction. However, since more calcium than sulphate is lost both by leaching (16, 43, 56) and by crop removal (Table 2) tobacco soils tend to become more acid under excessive sulphating (59). Heavy applications of fertilizer materials such as nitrate of lime or barnyard manure may effect a considerable increase in soil pH (55).

Since black rootrot susceptible varieties are used to produce about 80% of the tobacco in Canada, the selection and maintenance of soils at pH's between 5.0 to 5.6 may be of considerable practical importance to growers of flue-cured and other types for which resistant varieties have not been developed. Cigar and burley resistant varieties are available and these have been grown on soils with much higher pH's than 5.6 as pointed out by Kightlinger (42) and Murwin (59) who obtained improved yields and quality of leaf. Further application of lime even proved beneficial for burley resistant varieties (59). The fact that plants absorb more calcium from the soil at pH 7.0 than at 5.0 (57, 66) may explain in part why flue-cured tobacco absorbs less calcium (87) than cigar or burley. The flue-cured susceptible varieties are generally grown on the lighter sandy soils having a more acid reaction.

PHOSPHORUS

Role in Plants

This element appears to be necessary for all forms of plant life. It is a constituent of several very active plant compounds which are either enzymes or are associated with enzyme activity. Such substances are nucleoproteins, nucleotide enzymes, hexosephosphates, phytin and phospholipids all contain phosphorus. Nucleoproteins function in cell division.

Phytin is a storage form of phosphorus which may be released for plant use by the enzyme phytase. Hexosephosphates are important in carbohydrate metabolism; these compounds may be formed by the hexosephosphatase enzymes and subsequently hydrolyzed by phosphatase enzymes (54). The nucleotide enzymes are essential for the normal growth of tissue (65). There are 2 groups, viz., the pyridine and flavoprotein nucleotides. Coenzymes I and II belong to the pyridine group. The former is better known as cozymase, codehydrogenase I or diphosphopyridine nucleotide (72). The flavoproteins function in oxidizing the coenzymes (65). Thus, in enzyme systems, the mobile phosphate component may be used repeatedly and consequently only a small amount is required. Lecithin, an ester of glycerol, is a waxy hygroscopic solid and the best known phospholipid (34). It is present in practically all living tissue. Cephalin and sphingomyelin are other phospholipids. Phosphate is considered necessary for nitrate reduction and subsequent nitrogen metabolism (45). All of the above mentioned substances have not yet been identified in tobacco. Nevertheless, it seems reasonable to believe that they may exist at least at some stage of the tobacco plant growth.

Dry cured leaves from normal tobacco plants may contain from 0.4 to 1.0% of P_2O_5 (8). Phosphorus deficiency may resemble nitrogen deficiency in reducing the protein and increasing the amide contents of plants (1). Anderson *et al.* (8) and McMurtrey (51) have pointed out that: a lack of available phosphate in soil stunts the growth of plants, producing dark green, leathery, shiny leaves narrowed at the base; an ample supply of available phosphate accelerates the rate of cell division, effecting a general speeding up of plant growth and a rapid maturity of the leaves; properly matured leaves are essential for good curing and ultimate product of high quality.

Nutrient Relations of Cigar Tobacco

The phosphate quantities applied to and absorbed annually by Ottawa grown cigar tobacco are shown in Table 2. The basal ration of 160 lb. P_2O_5 per acre consisted of 120 lb. supplied by superphosphate and the remaining 40 lb. by cottonseed meal. An acre of cigar tobacco removed an average of 26.4 lb. P_2O_5 from the soil, equivalent to 1/6 of the total annual quantity applied. The optimum P_2O_5 content averaged about 0.5% of the dried cigar leaf (87); from other plots the content ranged from 0.21 to 0.75%.

In spite of the small phosphorus utilization by tobacco, a large excess has necessarily been applied to field soils to allow for the rapid fixation of phosphate and to ensure that enough remains in available form for optimum nutrition. At Ottawa, experiments are now in progress to study cigar tobacco yield and quality resulting from superphosphate applied at the rate of 80 lb. P_2O_5 per acre on plots which formerly received superphosphate at rates of 0, 40, 120, and 280 lb. P_2O_5 per acre for 14 years.

Soil Reactions and Phosphate Fixation

Doran (28) found with tobacco pot experiments that soil acidity was increased by a solution of phosphoric acid from pH 5.9 to 5.0 and that both the black rootrot and yield increased. These experiments apparently

were not duplicated in the field; however, beneficial results were obtained in the field by solutions of phosphoric and sulphuric acids combined, the soil pH was lowered and the damage from black rootrot was reduced. Monocalcium phosphate had little effect in reducing soil pH. According to Morgan *et al.* (55), superphosphate, precipitated bone, bone meal and steamed bone all made the soil slightly *less acid.*

The adsorption of phosphate ions by soil colloids (11, 12, 13, 14, 41) is known as "phosphate fixation" (26, 38, 58, 65, 68, 88). It is responsible for the rapid disappearance of a large proportion of available phosphate added to "acid" soils. When "fixed" it is not sufficiently utilizable for the optimum growth of most crops. As no appreciable quantity of phosphate is lost by leaching (16, 43, 56), the excess amount applied annually may be accumulated chiefly either in the ploughed soil layer or within reach of the plant root system in an unavailable state (21, 35). Investigators further indicate that phosphate may remain fixed until released by the action of micro-organisms (86, 88) and/or by biologically active organic matter (22, 23, 86, 88) or in acid soils by added lime (33, 53, 68, 80, 88). There is some evidence of a phosphate-humus complex which may be available to plants (22, 23). It is not economically feasible to satisfy the phosphate fixing capacity. The saturation capacity of a sandy loam soil has been established at 5100 lb. P_2O_5 per acre (37). Volk (85) concluded that the phosphorus not accounted for in the top 16 in. of a fine sandy loam soil was lost by erosion.

Excess phosphate when fixed in soil appears to be no more and sometimes even less available to most plants than natural rock phosphate (21). Conversely, some plants may possess root systems capable of utilizing either fixed or rock phosphate, viz., cabbage (61), potatoes (18), buckwheat, rape and white mustard and peas (25, 78), crested wheat grass, beans and turnips, at least in some climates. In Connecticut on old tobacco soils, Brown (18) obtained the same yield in 5 successive potato crops from a 5–0–6 as from a 5–8–6 fertilizer mixture. Crested wheat grass and beans, which apparently do not respond to available phosphate fertilizer, are reputedly better able than most crops to obtain this nutrient from the natural soil phosphate (35). Wright of the Chemistry Division, Ottawa, found that turnip yields from finely ground rock phosphate (Ephos) compared favourably with those from superphosphate and basic slag, but mangels and other crops did not respond to ground rock phosphate; these experiments (73) were conducted in the maritime provinces of Nova Scotia, Prince Edward Island and British Columbia. Truog (79) found buckwheat, rape, white mustard, clover and alfalfa all had strong feeding powers for rock phosphate and suggested that they be used as cover and green manuring crops in a rotation system; phosphate might thus be made available for the succeeding crop and also the acid formed from decaying organic matter might make still more phosphate available.

SULPHUR

Role in Plants

Considerable attention has been given recently to the sulphur nutrition of field crops (20, 29, 36, 46, 89). It appears that this nutrient element is a more important plant food than formerly considered. The amino acids cystine and methionine and the peptide glutathione (34) contain sulfhydryl

groups which may function as activators and inhibitors of a number of plant enzyme systems (67); thiamine (vitamin B_1), containing about 10% sulphur, is important also in plant enzymatic relations. The volatile essential oils of plants are sulphur compounds. Under conditions of excess supply, plants may take up more sulphate than is needed physiologically (54) and accumulate it in the cell sap as inorganic sulphate (10, 31, 40).

The optimum organic sulphur content of tobacco is very small, 0.15% of the leaf dry matter (10). According to Anderson *et al.* (8, 10), tobacco normally contains about 0.5% S (1.2% SO_3); tobacco soils are seldom deficient in sulphur; the sulphur requirement of tobacco is so small that rainfall supplies sufficient from the atmosphere and extra sulphur need not be applied in fertilizers. Garner (31) found that sulphates of bases especially of potassium were readily extracted with water from ground dried tobacco and he concluded that much valuable potash was thus "tied up" in the plant. Excessive absorption of sulphate has been found to adversely affect the quality of cigar leaf in Connecticut (10, 76). In view of the pale green coloured blades and white veins of sulphur-deficient leaves as produced by greenhouse experiments, Anderson, Swanback and Street (8) concluded that sulphur may be of some importance in plant production of chlorophyll, although not essential for chlorophyll formation. The pale green sulphur-deficiency colour showed up more in young than in old leaves. McMurtrey (51) suggests that sulphur deficiency may be advantageous in producing a more desirable flue-cured leaf colour, while an excess of sulphur in Maryland type of tobacco may result in a better cured leaf colour, providing the supply of other nutrients is adequate.

Nutrient Relations of Cigar Tobacco

Table 2 presents a rather surpising picture of the quantity of sulphates supplied to basal fertilizer plots for cigar tobacco at Ottawa. A considerable quantity of sulphur was absorbed by cigar tobacco, viz., 55 lb. SO_3 per acre. The leaf averaged about 2.5% SO_3 (87). It is significant that an excess of about 400 lb. SO_3 has been applied annually for 17 years without apparent adverse effects to crop yields. It is notable that nearly twice as much sulphate was supplied by sulphate of ammonia alone as was absorbed annually by the crop. The sulphur content of cigar tobacco from Ottawa plots receiving different sulphate applications has ranged from 0.25 to 3.3% SO_3 of the dried leaf (87). Unpublished investigations indicate that luxury absorption of sulphate may be responsible for reducing the burning quality and grade index of tobacco grown on heavily sulphated soil. McEvoy (47) found in optimum nutrition studies that a relatively low concentration of SO_4 ions was required for the growth of flue-cured tobacco plants.

Fertilizer Carriers of Sulphate

Sulphate of Ammonia.—As early as 1927, Anderson, Nelson and Swanback (5) reported that sulphate of ammonia seriously lowered the fireholding capacity of cigar tobacco from both leaf strip and cigar burn tests. Later, Swanback and Anderson (76) found that sulphate of ammonia compared to other nitrogen sources produced darker, thicker leaves with

more prominent veins, and a cigar inferior in taste and aroma with a shorter burn and darker ash. Sulphate of potash appeared to be less objectionable but nevertheless was conducive to excessive sulphate absorption by tobacco (10, 32).

Calcium Sulphate.—A moderate use of calcium sulphate may be beneficial. Gypsum, a hydrate of calcium sulphate, applied at the rate of 250 to 500 lb. per acre definitely increased the yield and grade index of cigar tobacco in Connecticut (7); higher quantities adversely affected the quality more than the yield of cured leaf; the sulphate content increased and the fire-holding capacity decreased regularly, but not proportionately, by increasing quantities of gypsum. Superphosphate, applied at the rate of 80 to 120 lb. P_2O_5 per acre, would appear to be in itself an ample source of sulphate for cigar tobacco. Hill and Hendricks (39) found that the water-soluble sulphate in commercial superphosphates ranged from 10 to 15% SO_3.

Sulphate of Potash.—Potassium is included in Table 2 in order to make a comparison with the major nutrients present in superphosphate. Even at the large annual rate of application of 200 lb. K_2O per acre, potassium was significantly different to phosphorus, sulphur and calcium, in that more potash was absorbed by the crop than was applied to the soil. With calcium and potash supplied to sandy loam soils at the rates shown in Table 2, there is no danger of a potash deficiency in cigar tobacco. This may confirm the view that a high concentration of potassium ions in the soil may depress the calcium ion absorption more than calcium depresses the potassium absorption as pointed out by Pierre and Allaway (66).

At Ottawa, most of the potash and 1/5 of the nitrogen have been applied to basal-diet plots as sulphate salts. Sulphate also has been applied in superphosphate. Table 2 shows the sulphate quantities from the 3 fertilizer sources. It is apparent that sulphate was thus excessively applied. The sulphates of ammonia and potash, for experimental plots, might readily be replaced by nitrate of ammonia and carbonate of potash. For high quality cigar tobacco the chloride of potash is prohibitive. The silicate or carbonate of potash are considered ideal for tobacco but no cheap commercial process has yet been developed for their production (31). It has been largely a matter of choosing between the chloride and sulphate commercial sources of potash and the latter has been less objectionable. However, the use of nitrate of potash in commercial NPK mixtures, in quantity to provide the nitrate proportion, would reduce the sulphate content substantially by replacing part if not all of the sulphate of potash.

Soil Reactions

Comparatively little is known regarding the fate of sulphates added to soils. A considerable amount is undoubtedly lost by leaching (16, 43, 56) where annual precipitations are in excess of 25 in. Some sulphate may be adsorbed by soil colloids (15) and consequently retained in the soil. The bulk of sulphur in a podzol soil has been found by Evans and Rost (29) to be equally distributed between the organic form and the non-sulphate inorganic form and they concluded that soil organic matter acts as a reservoir for sulphur. In Connecticut (76) long continued large annual applications of sulphate of ammonia increased the soil acidity to such an

extent that tobacco would not grow; the mineral bases of the soil became depleted; the nitrate and available phosphate decreased and the concentration of ammonium, aluminum and manganese ions increased. In wet seasons, sulphating of soils low in magnesium reserve may result in magnesium deficient tobacco. Garner *et al* (30, 32) found that sulphate of potash, compared to the chloride, not only produced lower tobacco yields but also symptoms of magnesium deficiency. Later Anderson *et al.* (76) confirmed this magnesium-hunger chlorosis of cigar tobacco on sulphate of ammonia plots and corrected it by increasing the magnesium supply.

The use of acid producing fertilizers, to decrease the soil pH from 6.0 to the optimum reaction range of 5.6 to 5.0 for growing tobacco varieties susceptible to black rootrot, has been recommended in Connecticut (55). Sulphate of ammonia appeared to be the quickest and most effective but the most detrimental to tobacco. Annual applications of sulphates of potash and calcium also apparently tend to make tobacco soils more acid in reaction because more basic ions than acidic ions are removed from the soil by leaching rainfalls and continuous cropping. According to Murwin (59) the lighter soils in Ontario have gradually become more acid after a number of tobacco crops have been grown.

NUTRIENT VALUE IN TERMS OF POUND-EQUIVALENTS

Table 3 contains the ionic and ionic-equivalents values corresponding to the data of Table 2. Pound-equivalents, viz., the ionic pound weight divided by its chemical equivalent weight, are used in an attempt to arrive at a common basis for appraising the value of phosphate, sulphate and calcium nutrients.

The absorption values of Table 3 are of particular interest when the 3 nutrients supplied by superphosphate are compared with potassium. The pound-equivalents value of calcium, utilized by cigar tobacco, is nearly equal to that of potassium, is more than 3 times that of sulphate (SO_4) and 12 times that of phosphate (H_2PO_4) ion. This basis of values would seem to give to calcium a high rating as a nutrient for plant growth as well as an important neutralizer of excess and harmful organic acids metabolized in the plant. Also in Table 3, it is notable that 4 times as many equivalents of SO_4 ions as H_2PO_4 were absorbed. Considering the small quantity absorbed, viz., 0.37 pound-equivalents H_2PO_4 per acre, the chief role of phosphorus, in promoting rapid growth of tobacco plants, appears to be catalytic by virtue of its enzyme compounds or its association with enzyme systems.

TABLE 3.—POTENTIAL POUND-EQUIVALENT OF NUTRIENT IONS SUPPLIED TO, AND ABSORBED ANNUALLY BY, CIGAR TOBACCO

(Converted from data of Table 2)

(Ionic values in lb. per acre are given in brackets)

Nutrient ion		Pound-equivalents per acre	
		Applied to soil	Absorbed by crop
Phosphate	H_2PO_4	(219) 2.27	(36) .37
Sulphate	SO_4	(546) 11.4	(66) 1.38
Calcium	Ca	(150) 7.5	(88) 4.4
Potassium	K	(166) 4.27	(196) 5.0

PHOSPHATE ABSORPTION

The monovalent ion, H_2PO_4, has been used arbitrarily in Table 3 to express phosphate nutrient value. A brief physico-chemical consideration of the dissociation of phosphoric acid into its successive ions may justify to some extent its use in tobacco plant absorption data. The following equation is a variant of the law of mass action:

$$pH = pK + \log \frac{(HPO_4^{--})}{(H_2PO_4^{-})}$$

The brackets designate the concentration of the mono- and di-valent phosphate ions. The value of the constant term pK, viz. the logarithm of the reciprocal of the dissociation constant of the second H of H_3PO_4, is 6.85. Accordingly in the equation,

$$pH = 6.85 + \log \frac{(HPO_4^{--})}{(H_2PO_4^{-})}$$

when the ionic concentrations of HPO_4 and H_2PO_4 are equal, the value of the log factor is zero and the pH = 6.85. Above or below pH 6.85, both ions are present but in different proportions according to the equation. At pH values below 6.85, the concentration of H_2PO_4 ions exceeds that of HPO_4 ions. As shown by Buehrer (19) in a phosphate solution (1 p.p.m.), at pH 4.5 all the phosphate is in the form of H_2PO_4 ions; at pH 6.0 the concentration of H_2PO_4 ions is only about 5 times greater than that of HPO_4 ions, viz., a ratio of 5 : 1.

However, the pH value which a plant is capable of inducing at the proximity of its roots and the pH of the plant sap (81-84), both undoubtedly govern to a large extent the dissociated form of phosphate ions taken up by the plant. According to Truog (84), carbonic acid excretions of some plants are capable of producing a pH value of 4 at the point of contact between root hairs and soil particles. At the range of pH 5.0 to 5.6 in "acid" tobacco soils, the existing proportion of H_2PO_4 ions for plant absorption is very high and that of HPO_4 ions very low. These H_2PO_4 ions are supplied by monocalcium phosphate and consequently superphosphate has been found to supply phosphate in a readily available form for tobacco crops. In addition, as previously mentioned, a dry, hot season may increase soil acidity to the extent of lowering the soil pH by as much as 0.3 to 1.0 unit (9), so that soils testing pH 6.0 in April or October, may react as low as 5.7 to 5.0 in June, July or August.

In neutral and alkaline soils with pH values above 6.85, a greater proportion of HPO_4 ions predominate and dicalcic phosphate is considered to be a good source of available phosphate. However, in soils containing available phosphate, within the pH range of ordinary plant growth both H_2PO_4 and HPO_4 phosphate ions exist but in different proportions except at a pH of 6.85 when their concentrations are equal, as pointed out by Buehrer (19) and McGeorge *et al.* (48, 49). These investigators and Breazeale (17) concluded that carbonic acid exudations of root systems and soil pH were important factors in converting HPO_4 to H_2PO_4 ions and that H_2PO_4 ions were preferred if not demanded by plants.

CONCLUSION AND SUMMARY

This paper includes a literature study of the composition and nutrient value of superphosphate for tobacco. Some aspects of the individual roles of calcium, phosphorus and sulphur are reviewed and discussed in respect to plant growth, yield and quality of tobacco, and soil reaction. The quantities of these 3 major nutrient elements applied to and absorbed by a crop of cigar tobacco at Ottawa are tabulated as oxides, ions and ionic-equivalents in pounds per acre and compared with the corresponding quantities of potassium. A variant-equation of the law of mass action is employed to show that most of the phosphate absorbed by plants is probably in the form of (H_2PO_4) ions in the majority of Canadian tobacco soils.

Commercial superphosphate, 16 or 20% P_2O_5 grade, contains more calcium and sulphur than phosphorus, about 28% CaO and 26 to 29% SO_3. These 3 nutrient elements are important factors in nitrogen and carbohydrate metabolism of tobacco. Phosphorus and sulphur compounds function chiefly either as enzymes or in enzymatic systems with characteristic catalytic effect and consequently only small quantities are needed by the plant for normal growth. Calcium functions chiefly in cellular structure and as a neutralizer of harmful organic acid-products of plant metabolism and is required in comparatively large quantities.

These nutrient requirements are confirmed by plant absorption data of calcium and phosphorus in particular when converted to potential ionic-equivalents. The pound-equivalents value of calcium ion absorbed by an acre of cigar tobacco at Ottawa was nearly equal to that of potassium, 3 times that of sulphate (SO_4) and 12 times that of phosphate (H_2PO_4). About 8, 6 and 2 times as much sulphate, phosphate and calcium, respectively, were applied annually in a basal fertilizer ration as were absorbed by the crop.

The excessive sulphate supplied in a cigar tobacco basal fertilizer ration is a problem for future adjustment. The consequent luxury absorption of sulphate by the tobacco plant and the adverse effects on cigar leaf quality are discussed. As a contributory factor toward quality improvement it may be necessary to replace the sulphates of ammonia and potash by nitrate of ammonia and carbonate of potash in plot experiments. Some progress has been made in recent years on Ottawa plots toward reducing the sulphate quantity from the superphosphate source by 1/3.

Phosphate is necessarily applied in excess to allow for phosphate fixation and to ensure rapid growth during the short growing season. Phosphate fixation in soils is also a problem requiring further investigation. Other crops, cover and green manuring crops, capable of utilizing fixed phosphate with the possibility of making soil phosphate more available to tobacco are discussed.

It is significant that the high calcium requirement of most Canadian tobacco crops has been supplied amply by the use of superphosphate in fertilizer mixtures. Soil pH is discussed in connection with lime requirement and the possible control of black rootrot disease of susceptible tobacco varieties, especially of flue-cured and other types for which resistant varieties are only now being developed.

An adequate and effective source of calcium and sulphate as well as phosphate and iron nutrients for tobacco exists in superphosphate. The necessity of providing for calcium, sulphur, and also magnesium, boron and manganese *in the proper proportions* in commercial NPK fertilizer mixtures for tobacco has been indicated by recent investigations.

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PASTURE STUDIES XXVII: THE EFFECTS OF MATURITY OF THE PLANT AND ITS LIGNIFICATION AND SUBSEQUENT DIGESTIBILITY BY ANIMALS AS INDICATED BY METHODS OF PLANT HISTOLOGY[1]

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The total amount of lignin and its mode of deposition in the stems of cured hay has been reported to play a role in determining digestibility and nutritive value of the forage. The evidence, however, is meagre and further data concerning the nutritional significance of lignin is needed.

Botanists consider lignin as that group of substances which during plant growth impart stiffness and greater durability to the cell wall. Clarke (1) regards the cell wall as a continuous interpenetrating system of cellulose and lignin and he likens the structure to reinforced concrete in which the iron rods represent the cellulose framework and the concrete the lignin and other constituents.

The chemist contends that lignin isolated from plants is not a single compound but a group of somewhat similar organic substances. These contain methoxyl and phenolic groups, aromatic nuclei and a carbon-oxygen ratio higher than that of true carbohydrates. However, the exact structure of lignin remains obscure and consequently its quantitative determination is still somewhat empirical.

The animal nutritionist finds that lignin in forage crops is not only useless as a nutrient, but may have an adverse effect upon the availability of nutrients contained within lignified cells. Comprehensive reviews of the literature on lignin acting as a physical barrier to the activity of microflora and fauna of ruminants in digesting cellulose are given by Watson and Horton (8), Patton and Gieseker (7), Clayson (2), Norman (6) and Maynard (5).

Data as to the digestibility of lignin are variable. This is not surprising since the methods for lignin isolation have not been perfected. Considerable work has been done at Macdonald College on the intra-seasonal changes in nutritive value of pasture herbage as influenced by lignin content (3 and 4). While large variations in digestibility coefficients existed between clippings, representing herbage grown at various times of the year, the chemical analyses showed no difference of sufficient magnitude in any constituent except lignin to account for changes in digestibility.

MATERIALS AND METHODS

In order to study the mode of deposition of lignin and the effect thereof on digestibility, experiments were started in May, 1944 using methods of plant histology to trace the process of lignification in red clover plants cut at five different periods of maturity. The degree of lignification was determined for each stage, and the findings related to cell digestibility as observed microscopically in the fecal residues.

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TABLE 1.—SHOWING STAGES OF MATURITY AND DATES AT WHICH THE CLOVER PLANTS WERE CUT

Stage of maturity	Cutting No.	Date of cutting	
		1944	1945
Early vegetative	1	May 26	June 1
Early bud	2	June 1	June 8
One-third flowering	3	June 12	June 21
Full bloom	4	June 22	June 30
Heads browning	5	July 10	July 12

TABLE 2.—RATIOS OF THE DRY MATTER COMPOSITION OF THE VARIOUS FRACTIONS OF THE RED CLOVER PLANT CUT AT 5 DIFFERENT STAGES OF MATURITY (DENOTED BY CUTTINGS) DURING 2 DIFFERENT YEARS

Plant fraction	Year	Cutting 1	Cutting 2	Cutting 3	Cutting 4	Cutting 5
		Early vegetative	Early bud	One-third flowering	Full bloom	Heads browning
Blossoms	1944	0	3.1	12.4	16.5	25.5
	1945	0	0	4.6	9.4	12.6
Leaves	1944	46.4	33.8	24.4	22.0	18.4
	1945	39.3	34.9	25.6	19.1	13.9
Stems + buds	1944	53.6	63.1	63.2	61.5	56.1
	1945	60.7	65.1	69.8	71.5	71.5
Leaf stem ratio	1944	.86	.56	.56	.63	.78
	1945	.65	.54	.43	.39	.39

A field of Dollard red clover at Macdonald College farm provided the material for the first series of trials. The herbage, following cutting, was placed at once in a Wisconsin-type corn drier where a temperature of approximately 43° C. was maintained for 48 hours. After drying, it was chopped in a silage cutter, bagged and stored until ready for feeding and lignin analysis. In 1945, samples of clover were again obtained but one difference in curing should be noted, namely, that the herbage was field cured on tripods instead of being dried in the corn drier. No feeding of 1945 forage was done.

At the time of making each cutting, several entire plants were uprooted and while still in the succulent state, separated into blossoms, leaves, stems and buds. These fractions were then dried at 218° F. in the oven and relative weights of the plant fractions determined.

Also at cutting time, some 2 doz. representative plants were selected from the sward and a typical stem selected from each plant for microtoming. A $\frac{1}{4}$ in. portion of this stem was taken above the first internode (mower height) and another $1\frac{1}{2}$ in. from the apex of the shoot, in order to study the development of all types of cells in these particular regions of the stem. The stems were fixed in Craf II. In the summer of 1945, areas of the stem mid-way between the top and bottom of the stem axis were also taken.

MICROTECHNIQUE

Microtoming of the paraffin embedded stems presented many difficulties because of hardness and toughness of the lignified vascular strands. The ultimate procedure developed will be explained. All sections were cut at 10 microns on a rotary microtome, and stained with haemotoxylin and safranin—the positions of the lignified cells being first corroborated with phloroglucinol.

Preparation of Clover Stems for Microscopic Examination

Portions of the stem about $\frac{1}{4}$ in. in length were obtained from the selected stems by cutting with a razor. The $\frac{1}{4}$ in. sections were placed individually in vials containing a freshly prepared fixative Craf II. The vials containing the stem sections were taken to the laboratory where they were subjected to vacuum pressure to withdraw the air from the intercellular spaces. This aided in the penetration of the fixative and the prompt killing of the cells with a minimum of plasmolysis. After pumping for about $\frac{1}{2}$ hr., the vials were corked and stored until ready for embedding.

Infiltration was carried out in the following steps: Dehydration in an ethyl alcohol series starting with 10% and progressing in 10 steps to 100% ethyl alcohol. Sections were then introduced into 25% xylol, transferred to 50% xylol, then to 75% and finally to pure xylol. Ten ml. of xylol were introduced into a 50 ml. beaker and the stems placed therein with enough 52° tissuemat to make the xylol come up to about the 20 ml. mark. The beakers were placed in an oven at 55° C. and allowed to heat for 1 hr., after which small pieces of tissuemat were added intermittently every 30 min. to increase the concentration of tissuemat as infiltration progressed. After 4 hours the xylol-tissuemat mixture was poured off and fresh molten tissuemat added and again allowed to stand for 4 hr. at 55° C.

The sections were then embedded in tissuemat of 60° melting point.

Three days before microtoming these paraffin blocks were put into water at about 45° C. and allowed to soak for 36 hr. This last step was found highly desirable to soften the stems for microtoming.

After the paraffin blocks were trimmed to proper size, they were fitted to wooden blocks which would conveniently fit into a microtome clamp. Cross and longitudinal sections 10 microns in thickness were cut with a rotary microtome.

Staining and Processing of Slides

The microtomed sections were fixed to microscope slides by using Haupt's adhesive and this staining schedule followed:

Xylol—100% ethyl alcohol (1 : 1)
100% ethyl alcohol
95% ethyl alcohol
90% ethyl alcohol
70% ethyl alcohol
50% ethyl alcohol
30% ethyl alcohol
10% ethyl alcohol
Distilled water.

Mordant in 3% iron alum for 1 hr.
Rinse in distilled water and wash in tap water 1 hr.
Iron haematoxylin, 1 ml. of a .5% aqueous stock solution plus 1 drop NH_4OH in a Coplin jar full of distilled water.
Stained for 15 sec.
Rinse in tap water.
Safranin, 5 drops of a 1% stock solution in 50 ml. of tap water.
Stain for 4 hr.
50% ethyl alcohol (30 sec.).
70% ethyl alcohol (10 sec.).
90% ethyl alcohol (10 sec.).
95% ethyl alcohol (10 sec.).
100% ethyl alcohol (15 sec.).
100% ethyl alcohol (15 sec.).
Xylol-carbol (3 : 1) (3 min.).
Xylol 1 (5 min.).
Xylol 2 (5 min.).
Xylol 3 (5 min.).
Mount in balsam.

COLLECTION OF UNDIGESTED RESIDUES

To obtain samples of undigested portions of the forage and of urine from these diets, 3 cheviot wethers were confined in metabolism cages designed to permit the collection of feces and urine.

By careful washing, separation and sampling, it was possible to prepare slides showing the character of the undigested portions of the plants of different maturities.

Microscopic Examination of Fecal Residue

Preparation of slides for microscopic examination of the fecal residues was as follows: Two feces pellets were placed in a 10 ml. test tube and about 2 ml. warm water added, after which they were macerated gently with a rubber policeman. After some disintegration had occurred, the test tubes containing the water-feces suspension were centrifuged at 1200 r.p.m. for ½ min. The liquid was decanted and the process repeated until separate plant cell entities were obtained. These washed residues from each of the cuttings were separately bulked and oven dried at 50° C. Two hundred mg. samples of each of the 5 fecal residues were again dispersed as above and then stained with aqueous safranin. As the slides were to be made into permanent mounts, the test tubes containing the stained feces were brought up through the ethyl alcohol series, each change being accompanied by centrifuging. The residues were finally terminated in pure xylol and the whole suspension made up to a volume of 10 ml. This was transferred to a plastic centrifuge tube and swirled 20 times to distribute the cells. Five drops of the suspension were then transferred to a slide by using a medicine dropper-like device consisting of a piece of pyrex glass tubing 3 in. long and 3/16 in. in cross section, outfitted with a rubber bulb and so arranged that when the bulb was completely squeezed and then released, the tube would withdraw 5 drops of the fecal-xylol suspension. Excess xylol on the slide was soaked up with filter paper

and 3 drops of balsam added to the sample. Both cells and balsam were thoroughly mixed with a warmed dissecting needle in order to obtain an even distribution of cells under the cover slip.

RESULTS

At the outset it may be stated that these studies indicate that lignification proceeds regularly with advancing maturity of the plant and that regions around the vascular bundles are primarily involved in this process. In this paper, slides of cutting 1, early vegetative stage, and of cutting 5, heads browning stage, will be used to illustrate the findings.

In Plate I, photomicrographs (× 100) show the difference between the part of the stem and the age of the plant in relation to lignification. Xylem is the first tissue in the stem to undergo lignification. This begins in the bottom ends of the stem. With increasing maturity this complex xylem tissue becomes more fully developed and lignified throughout the whole length of the stem. The xylem at maturity consists of the annular and spiral vessels of the primary xylem and also the pitted vessels of the mature tissue. The xylem parenchyma also become lignified with maturity and the food reserves of these cells, therefore, unavailable to the animal.

Sclerenchyma is a tissue formed by the transmutation of the parenchyma cells. The walls of these cells become thickened through lignification as the plant matures. It will be noted that sclerenchyma is absent from the upper stem areas in the immature plant.

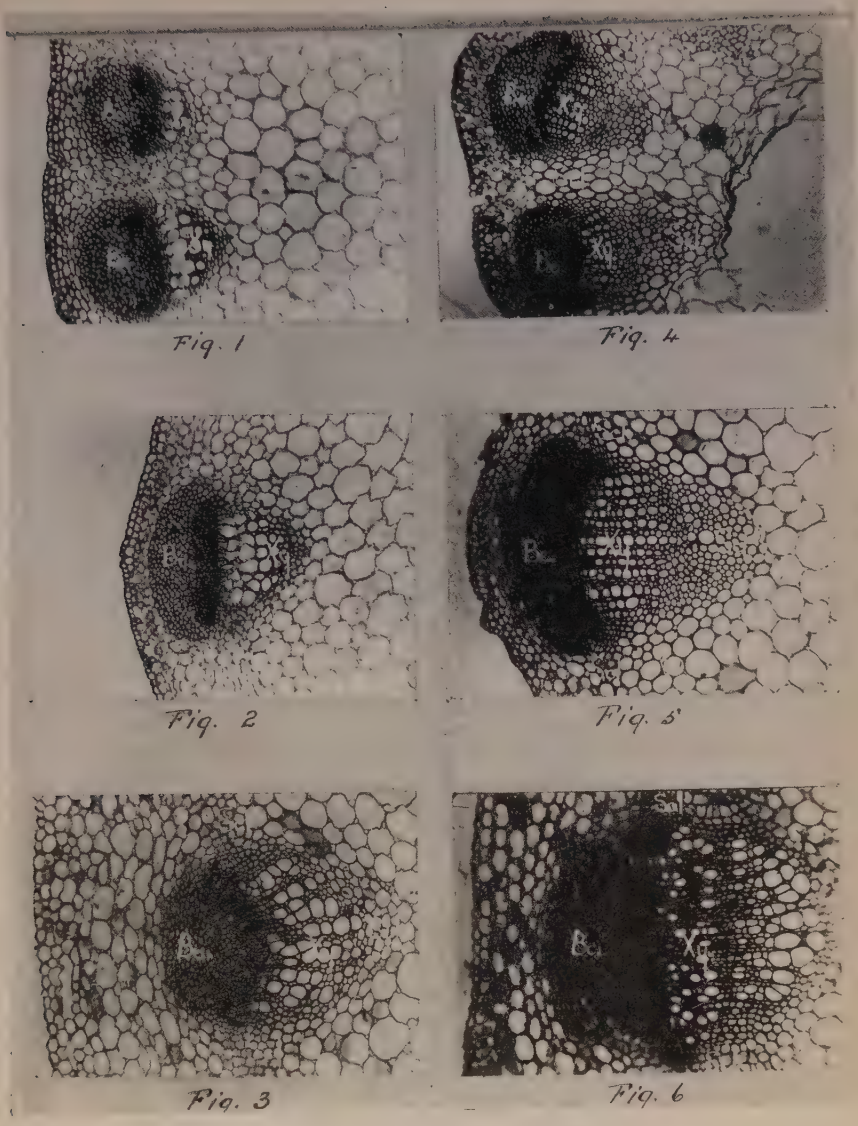
In the very young plant stem, the bundle cap consists of thin walled living cells in which the cell contents are plasmolized. In the mature stem the protoplasm disintegrates and a heavily lignified secondary wall develops on the cell. These changes in the cells of the bundle cap are shown in greater detail in Plate II.

Some indication of the rate of lignification in terms of the entire plant may be had from the data for percent lignin by chemical analysis of the several clippings as given in Table 3.

TABLE 3.—SHOWING THE PERCENT LIGNIN FOUND IN THE 5 CUTTINGS OF RED CLOVER DURING 2 DIFFERENT SEASONS

Cutting No.	Lignin content in %	
	1944	1945
1	7.3	7.6
2	7.7	10.1
3	7.9	11.9
4	9.7	12.9
5	12.2	15.2

Plate III consists of 5 photomicrographs (× 50) representing the fecal residues of the 1944 forage from each of the 5 stages of maturity of the plants. It should be noted that the particles identified as pitted vessels (Pv) are parts of the xylem tissue. They are abundant in mature xylem but largely absent from the primary xylem of the young immature stem.



Photomicrographs (× 100) of Transverse Stem Sections.

FIGURE 1. Top segment, 1st cutting. The bundle cap (Bc) is thin walled and the xylem (Xy) is not yet well developed.

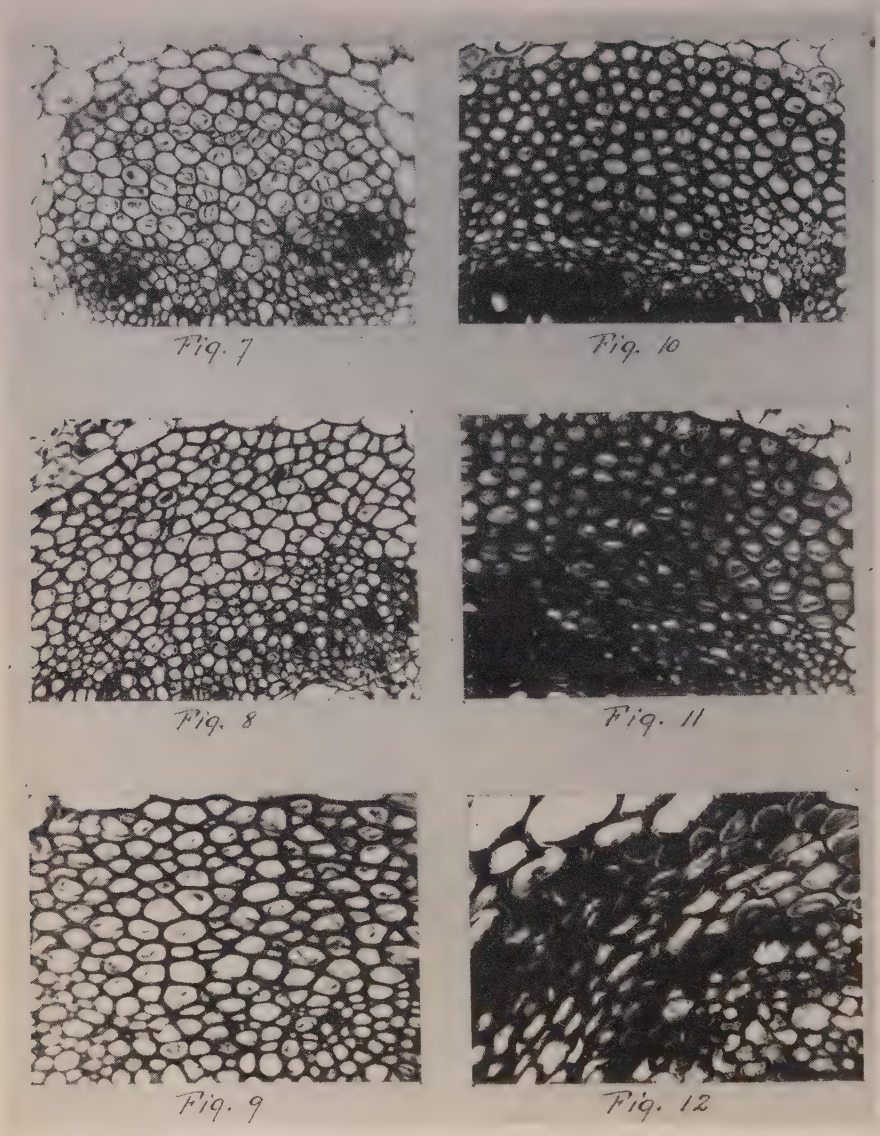
FIGURE 2. Middle segment, 1st cutting. Bundle cap is still thin walled but xylem shows some lignification of the cell walls.

FIGURE 3. Bottom segment, 1st cutting. Cell walls in the xylem are lignified. Sclerenchyma (Scl) is present around the bundle cap. Bundle cap cells are still thin walled.

FIGURE 4. Top segment, 5th cutting. The bundle cap at this stage consists of cells with lignified secondary walls. Xylem is well developed and somewhat lignified. Some sclerenchyma is present.

FIGURE 5. Middle segment, 5th cutting. Bundle cap and xylem cells are lignified.

FIGURE 6. Bottom segment, 5th cutting. Heavy lignification of bundle cap and xylem cells is evident. Sclerenchyma tissue surrounds the cap and xylem.



Photomicrographs (× 400) of Transverse Sections of Bundle Caps.

FIGURE 7. Top segment, 1st cutting. Cells are thin walled and protoplasts are seen within these living cells.

FIGURE 8. Middle segment, 1st cutting. No appreciable thickening of the cell walls is seen.

FIGURE 9. Bottom segment, 1st cutting. Cell walls are somewhat thickened and cell contents are decreasing.

FIGURES 10, 11, 12. Top, middle and bottom segments, respectively. Heavy walls and development of secondary walls are clearly shown. These walls are lignified.

PLATE III. Photomicrographs (× 50) of fecal residues recovered from sheep fed red clover forage of different maturities.

FIGURE 13. Cutting No. 1. The bundle cap is partially digested but the pitted vessel is intact.

FIGURE 14. Cutting No. 2. Bits of lignified bundle cap, sclerenchyma and xylem (pitted vessels) are present and more abundant than in Figure 13.

FIGURE 15. Cutting No. 3. At this stage the bundle cap was extensively lignified as was also the xylem.

FIGURE 16. Cutting No. 4. Sclerenchyma and pitted vessels of large particle size predominate. Numerous bundle cap fibres were also found in this sample.

FIGURE 17. Cutting No. 5. The large particle size, especially of bundle cap tissue is evidence of the heavy lignification in this cutting.

In general, the increase in the number of undigested bits of tissues and their increase in size is correlated with the increase in maturity of the forage eaten and its increasing lignification. The fecal particles shown in these pictures are all lignified and one might be justified in concluding that only lignified parts of the plant appear in quantity in the feces excepting substances which are encased by a lignified cell wall. Actually, the digestibility by sheep of the dry matter of the entire plants declined from 64% to 58% for forage, corresponding to cuttings No. 2 and No. 4, respectively.

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OBSERVATIONS ON THE USE OF FERRIC OXIDE AS A MARKER IN SWINE DIGESTION STUDIES[1]

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One of the major problems in conducting digestion trials is that of obtaining samples of feces that correspond exactly to the quantity of feed consumed. Investigators have from time to time developed techniques for eliminating or minimizing the errors involved. Generally the animals are subjected to pre-collection periods of varying lengths of time. For swine, the preliminary period has usually been at least 5 days, followed by a collection period of 10 days or more. Providing the animals are behaving favourably, there can be little doubt that this regimen will give reproducable results.

However, it is frequently necessary to obtain digestibility coefficients on rations for which this regimen is not readily applicable, either by reason of limited time, or because of the nature of the rations to be fed. Hence, it would be advantageous to have a system of feces collection which would minimize the need of consideration of previous diets, uniformity of feed intake and length of collection periods.

A considerable amount of work has been done with ruminants in studying iron oxide—and silica-ratio methods of determining digestibility. (Bergeim, 1926; Heller *et al.*, 1928; Knott *et al.*, 1936; Gallup and Kuhlman, 1931, 1936; Gallup, Hobbs and Briggs 1945). The principle is to use as an index some added or naturally occurring substance in the feed which is totally recoverable in the feces. The dry matter excreted as feces may be calculated from the following relationships:

$$\frac{\%\text{ index substance in dry matter of the ration}}{\%\text{ index substance in dry matter of the feces}} = \frac{\text{Wt. of dry matter in feces}}{\text{Wt. of dry matter in ration}}$$

Knowing from chemical analysis the percentage composition of ration and feces, the digestibility of any of the nutrients of the dry matter may then be calculated in the usual manner.

It is possible to use a modification of this system on those animals possessing simple stomachs, where it is easier to maintain the integrity of meals consumed several hours apart. Thus, if some coloured inert substance is fed with a meal, it should be possible to distinguish the residue of that meal from the one preceding it. The procedure consists of making quantitative feces collections between two such markers and attributing the amount of undigested material obtained to the known quantity of diet fed between the markers.

For several years ferric oxide has been used as such a marker in studies at this laboratory in connection with swine digestion trials. In this report, the results of marker collections have been compared with time collections.

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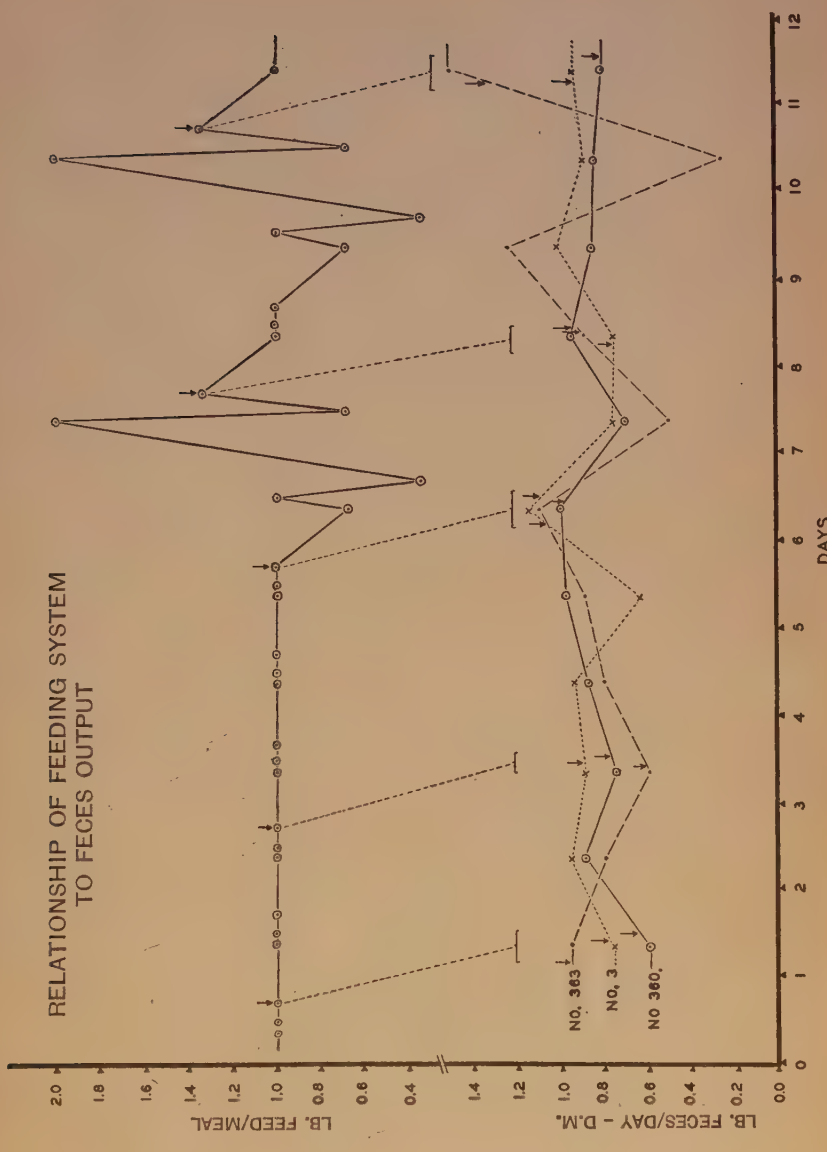

FIGURE 1. The position of the arrows shows where markers were fed and where they appeared in the feces.

EXPERIMENTAL—I

The animals used in the digestion studies herein reported were 4 Yorkshire barrows, averaging in weight 50, 100, 150 and 200 lb. at the beginning of each of the 4 tests. The rations studied in the first series of tests were corn, wheat, standard shorts[1] and degermed shorts.[2] Oats ground to 3 different moduli were fed during a second series of trials, designed to allow study of effects of feed intake, age of pig and length of trial on the reliability of marker collections.

Ferric oxide[3] was fed at intervals of 2 and of 3 days throughout the trials, so that comparisons could be made between trials of varying lengths on both methods of feces collection. The marker was fed at the rate of about ½ teaspoonful per lb. of feed. This was the minimum amount necessary to distinctly mark the feces.

The first appearance of the marker was always used as the division for feces collection. If the marker was fed with the evening meal, this division frequently came *within* a given defecation. It was found that this was largely avoided by feeding the marker with the morning instead of with the evening meal.

RESULTS AND DISCUSSION

At the outset it may be stated that difficulty was encountered in the use of markers with the Shorts rations. The markers were indistinct due to the natural colour of the feces from the shorts, and in addition the feces tended to disintegrate so that separation of portions was difficult and uncertain. Accordingly, data from the shorts rations have not been included in this report. The digestion coefficients obtained on the corn and wheat rations (Series I) are presented in full in Table 1 (a); those from Oat rations (Series II) in Table 2 (a).

In the trials of Series I, the digestion coefficients for the mixed rations were calculated for periods of 3, 5, 8 and 10 days' duration as separated by markers; and by 24 hour time intervals.

Table 1 shows the analysis of variance of wheat and corn coefficients according to methods of feces collection.

Of immediate interest is the fact that the use of markers has reduced the standard error, as well as the variance due to the lengths of collection periods over that of time collection. The fraction of the variance, however, was insignificant in both methods, meaning that the shorter trials, generally, were as reliable as were trials of 8 or 10 days' duration.

It was not possible to ascertain how much of the variance attributed to weights of pigs or to interaction of grains and weights was actually due to animal individuality. The results of a separate trial, however, involving the same 4 pigs on a uniform ration, indicated that most of the variability may have been due to differences between animals.

A direct comparison of the collection methods is presented in Table 2.

[1] Standard shorts consist of fine particles of bran, germ, and a small percentage of low grade flour as separated in the ordinary process of flour milling.

[2] Degermed shorts was a product similar to the standard shorts, except that it was a by-product of the Canada Approved flour, hence was lower in wheat germ content.

[3] Red hematite or native brown iron oxide.

TABLE 1.—ANALYSIS OF VARIANCE OF COLLECTION METHODS USING DIGESTION COEFFICIENTS FOR WHEAT AND CORN ON 10-, 8-, 5- AND 3-DAY TRIALS

Sources of variation	D/F	Time collections		Marker collections		Necessary F values
		Variance	F values	Variance	F values	1%
Grains (wheat vs. corn)	1	38.72*	27.08	34.65*	60.79	8.02
Weights of pigs	3	3.74	2.62	5.22*	9.16	4.87
Lengths of time	3	0.73	0.51	0.30	0.53	4.87
Grains × weights	3	8.10*	5.66	16.12*	28.28	4.87
Remainder	21	1.43	—	0.57	—	—
Standard error (σ)		1.19		0.75		

* Significant as indicated by F values.

TABLE 1 (a).—DRY MATTER DIGESTIBILITY COEFFICIENTS CALCULATED FROM 2 METHODS OF FECES COLLECTION IN SERIES I

Weight of pig, lb.	Length of trial in days	Corn		Wheat	
		Time	Marker	Time	Marker
50	10	81.5	80.7	81.7	81.6
	8	81.4	80.9	84.9	81.6
	5	84.0	82.1	82.8	82.3
	3	85.4	80.9	82.5	81.7
100	10	81.6	80.8	82.2	80.4
	8	81.6	80.7	81.9	80.6 (7)*
	5	82.0	80.7	81.0	79.0 (4)*
	3	82.6	79.8	78.4	80.3 (2)*
150	10	84.8	84.0	79.5	77.1
	8	85.0	84.9	79.9	78.0
	5	84.7	84.9	80.3	79.3
	3	83.6	84.4 (4)*	79.6	80.8
200	10	84.0	84.0	80.4	80.5
	8	83.3	83.8	80.5	80.8
	5	83.8	83.1	80.7	80.7 (4)*
	3	82.1	83.5	79.9	81.2

* Numbers in parentheses represent the number of days used to obtain the coefficient of digestibility shown. Other values are based on the number of days shown in column 2.

TABLE 2.—COMPARISON OF DIGESTION COEFFICIENTS ACCORDING TO TIME AND MARKER COLLECTIONS ON TRIALS OF VARYING LENGTHS. (FIGURES ARE % DIGESTIBILITY OF DRY MATTER AVERAGES OF 4 TRIALS)

Collection period (days)	Corn		Wheat	
	Time	Marker	Time	Marker
10	83.0	82.4	81.0	79.9
8	82.8	82.6	81.8	80.3
5	83.6	82.7	81.2	80.3
3	83.4	82.2	80.1	81.0
Necessary difference at P.05	1.3		1.3	

This table indicates that with carefully regulated constant daily feed intakes no advantage is apparent from the use of collection periods greater than 3 days long, and this regardless of whether time or marker separation is employed.

EXPERIMENTAL—II

In the second series of tests, similar spacing of markers was used, but the daily feed intakes were varied over the last 5 days of each trial, in order to study the effects of such variations on the efficacy of marker and time collections.

Figure 1 illustrates the feed intakes by feedings and the corresponding daily feces outputs for three 50 lb. pigs when fed a ration of finely ground oats. The first half shows typical variations in daily feces output when the pigs were on constant feed intakes, while the latter portions represent a continuation of these trials where the feed intake was varied by feedings and by days. The feed consumed over the 2 successive 5-day periods did not differ in total amount, so it was possible to compare collection methods on 5-day periods where one period had marked daily fluctuations.

The design of the second series of experiments allowed statistical analysis of the coefficients of digestion to determine effects of lengths of collection periods, age and individuality of pigs, and methods of feed intake, without any of the confounding inherent in trials of Series I.

Since the matter of fineness of grinding, and the factor of refused feed, have little bearing on the study of markers, data for one modulus only will be cited in this report. An analysis of the variability of the digestion coefficients for dry matter of the oat ration according to system of feces collection employed is given in Table 3.

It is evident from these data that there was no significant difference in the coefficients traceable to 2-, 3- or 5-day marker or 10-day time collections. However, it may be stated that the simple effect of ages becomes highly significant because of feed sorting on coarse oat rations by young pigs; and because of a decrease in digestibility of fine modulus oats when fed to older pigs. The differences between pigs indicated are to be expected because actual differences were emphasized by the replicating effect of having several collections from each animal.

A summary of the digestion coefficients according to collection system, method of feeding, length of collection period and age of pig is given in Table 4.

With reference to these data, it should be stated that time collections started about 16 hours after feeding the marker, except in the few trials where markers were fed with the morning meal. Since the time required for the digestion of oats was 15-20 hours, it is obvious that the two methods of collection should agree much better than if the time collection had begun several meals before or after the administration of ferric oxide. (See Figure 1).

It is apparent from Table 4 that a difference exists between collection methods with the lighter pigs; and from Table 5 it is seen that short periods during which feed intake is not constant are not satisfactory for such animals—a result which is to be expected in view of the difficulties encountered with young pigs in digestion crates.

TABLE 2 (a).—DRY MATTER DIGESTIBILITY COEFFICIENTS OF FINELY GROUND OATS ACCORDING TO METHOD OF FECES COLLECTION, LENGTH OF COLLECTION PERIOD, AND SYSTEM OF FEEDING, FOR VARIOUS WEIGHTS OF PIGS

Number and weight of pig (lb.)	Length of collection period (days)	Time collections		Marker collections	
		Constant feed intake	Varied feed intake	Constant feed intake	Varied feed intake
No. 1 50–60	2	66.9	72.0	67.2	74.1
	3	66.2	68.9	65.8	65.9
	5	66.4	67.9	66.4	69.1
No. 2 50–60	2	73.5	74.1	67.2	69.9
	3	72.1	67.4	66.7	66.7
	5	68.3	67.7	66.9	67.9
No. 3 50–60	2	69.1	68.9	69.7	67.1
	3	68.8	68.9	66.8	68.7
	5	66.7	68.8	68.0	66.9
No. 1 120–160	2	63.2	66.2	64.9	69.2
	3	63.1	67.3	64.5	65.4
	5	64.5	67.5	64.6	65.9
No. 2 120–160	2	65.1	65.9	65.5	67.5
	3	69.1	67.1	68.5	63.7
	5	67.5	65.2	67.3	65.5
No. 3 120–160	2	59.4	64.4	66.2	66.2
	3	63.9	65.8	65.0	66.5
	5	64.5	67.1	65.5	66.4

TABLE 3.—ANALYSIS OF VARIANCE SHOWING COMPARISONS OF 2-, 3- AND 5-DAY MARKER COLLECTION COEFFICIENTS WITH THE STANDARD 10-DAY TIME COLLECTIONS

Sources of variation	D/F	Variance	F values	
			Observed	Necessary 5%
Total	23			
Between ages of pigs	1	12.76	33.58	5.99
Between lots	2	3.55	9.34	5.14
Between lengths of time	3	0.55	1.45	4.76
Interaction				
Ages × lots	2	2.42	6.37	5.14
Ages × lengths of time	3	1.09	2.87	4.76
Lots × lengths	6	1.11	2.92	4.28
Remainder	6	0.38		

TABLE 4.—EFFECTS OF FEED INTAKE, LENGTH OF COLLECTION PERIOD, AND WEIGHT OF PIG ON DIGESTION COEFFICIENTS OBTAINED BY 2 METHODS OF FECES COLLECTION

Factor studied	Method of collection		Necessary difference (P = 0.05)
	Marker	Time	
Feed intake			
Constant	66.5	66.6	1.0
Varied	67.4	67.8	1.0
Collection period (days)			
2	67.9	67.4	1.2
3	66.2	67.4	1.2
5	66.7	66.8	1.2
Weight of pigs (lb.)			
50–60	67.8*	69.0*	1.0
120–160	65.4	66.0	1.0

* Indicates a difference greater than that necessary for significance at odds of 19 : 1.

TABLE 5.—EFFECTS OF FEED INTAKE AND WEIGHT OF PIG ON THE EFFICACY OF COLLECTION PERIODS OF VARYING LENGTHS AS SHOWN BY DIGESTION COEFFICIENTS

Factor studied	Length of collection period (days)			Necessary difference (P = 0.05)
	2	3	5	
Feed intake				
Constant	66.5	66.7	66.4	1.2
Varied	68.8*	66.9	67.2*	1.2
Weight of pig (lb.)				
50–60	70.0*	67.7*	67.6*	1.2
120–160	65.3	65.8	66.0	1.2

* Indicates a difference greater than that necessary for significance at odds of 19 : 1.

Other than for the younger pigs on fluctuating daily feed intake as noted above, the marker systems were equally as good as the time collections in these studies. The use of a marker such as ferric oxide appears, therefore, to be well adapted for use in digestibility trials with swine excepting with some feeds which because of their physical nature, do not lend themselves readily to the use of this marker.

CONCLUSIONS

The data from these experiments indicate that digestion trials on swine may be simplified by the use of feces markers.

Marker and time collections were equally reliable for 5- and for 10-day periods. Shorter periods, as a rule, favoured the marker system. Collection periods of less than 5 days were generally unsatisfactory for pigs of less than 100 lb. live weight, regardless of collection system.

Feces from corn, wheat and oats containing ferric oxide were readily distinguished from unmarked feces. Rations largely of shorts produced feces which disintegrated after voiding, and which were of a colour which prevented distinction of marked and unmarked material.

Variations in daily feed intake did not detract from the reliability of marker collections.

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THE INFLUENCE OF ACIDITY ON THE DEVELOPMENT OF LIPOLYTIC FLAVOUR DEFECTS IN CHEDDAR CHEESE[1]

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Both the extent and the course of action of milk lipase may be influenced under certain conditions. Dorner and Widmer (4) were the first to show that the addition of hydrochloric acid retarded or inhibited the development of hydrolytic rancidity in homogenized raw milk. Mattick and Kay (15) established the pH optimum for milk lipase between 8.2 and 8.7 which was confirmed by Roahen and Sommer (17) who reported a pH range of 8.4 to 8.6. These results were soon followed by more complete pH—activity studies first by Gould (6) and then by Peterson *et al.* (16). Gould found that lipolytic activity was affected adversely and permanently when milk was acidified and readjusted with alkali, even though exposed to the acid treatment for only a few minutes. He reported no appreciable lipolysis at pH 4 and 5. Peterson *et al.* found no lipase activity below pH 6.5. They also observed that readjustment of pH to 8.5 would restore only 30 to 50% of the original lipase activity of milk which had been held at pH 4.5 for 30 minutes at room temperature.

In their studies on the development of lipolytic flavour defects in cheddar cheese, Hlynka and Hood (7, 8) concluded from organoleptic data as well as from titratable acidities of cheese fat that milk lipase was inactive in cheese after it was removed from the press. Hydrogen ion concentration increases rapidly even during the early stages of manufacture as shown by Davis and Thiel (2) and by Irvine (10). According to Brown and Price (1) and Dolby *et al.* (3) the finished cheddar attains a pH of about 5.

That the course of action of milk lipase may be modified is indicated by several studies. Dorner and Widmer (4) observed that rancidity which developed in homogenized milk differed from that which developed spontaneously. They, therefore, suggested the existence of 2 lipases, the first attacking fat as a whole and the second resulting in predominantly volatile products. Krukovsky and Herrington (13) also postulated 2 lipases on the basis of formaldehyde tolerance while Gould (5) studied the difference in behaviour of lipase in homogenized and non-homogenized milk. Krukovsky and Sharp (14) reported "a depressing effect of the water soluble fatty acids produced by lipase activity, on further activity of lipase itself, and the establishment of a partition of fatty acids between the plasma and the fat which reduces the hydrolysis of the glycerides which produce the water-soluble acids. The lipase continues to hydrolyse glycerides which do not produce water soluble acids." Finally, Kelley (11, 12) showed that milk lipase appears to hydrolyse tributyrin and other short chain glycerides at a much more rapid rate than it does the longer chain glycerides.

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It was first indicated in our communication on the effect of agitation of cheese milk on lipase action in cheddar cheese (9) that the development of lactic acid in milk was a factor in the suppression of rancid flavour development. This preliminary observation has now been further examined in the light of the background outlined in this introduction.

EXPERIMENTAL

Experimental work was designed on a small manufacturing scale. Each vat of cheese was made from 160 lb. of Experimental Farm herd milk according to accepted method for the manufacture of Canadian cheddar cheese. Except where otherwise specified 3% of starter was employed. The curd was pressed into cheese weighing 10 to 12 lb. When removed from press, the cheese was held at 60° F. until the first grading, then placed in a 46° F. storage. It was returned to the higher temperature room for one day in order to attain a proper temperature for a second, and then again for a third grading. Grading was done by a senior member of the Dominion grading staff, Dairy Products Division.

A system of comparative vats was used. Milk to be used for an experiment was pooled and then divided between 2 vats. Both vats were then treated in the same way except for the factor which was to be studied. Any difference in the flavour scores of comparative cheese could then be attributed directly to the factor under consideration.

Acidification of Milk

To one of 2 comparative vats of morning milk concentrated hydrochloric acid was added dropwise, with stirring, until titratable acidity of 0.20% in terms of lactic was attained. In order to induce milk lipase action, 50% of the milk in each vat was homogenized at 86° F. using a centrifugal type homogenizer. When acidity in the unacidified vat reached 0.20%, hydrochloric acid in the acidified vat was neutralized with a stoichiometric amount of 8 N sodium hydroxide. Both vats were then set and the make proceeded with normally.

Grading results on cheese from several vats made on this basis revealed no significant differences between comparative cheese.

Accordingly, in the next set of experiments cheese milk was acidified with hydrochloric acid to a titratable acidity of 0.25% as lactic acid, and as previously this acid was neutralized quantitatively before setting. The proportion of milk homogenized in each vat remained the same. However, grading results still showed no significant differences between comparative cheese.

Since the time of exposure of milk to hydrochloric acid was only about an hour, further experiments were designed to increase the period of contact. Pooled morning milk was divided and one-half was acidified as before. All milk was then held in a cooling tank and cheese made next day, neutralizing the acidified vat with calculated amount of sodium hydroxide. Several vats were acidified to 0.20% and some to 0.25% expressed as lactic acid.

Grading results on comparative cheese showed a slightly higher flavour score for cheese from milk which had been acidified. A greater improvement was indicated in those vats which had been acidified to 0.25%. While these results were positive, the practical achievement was not considered sufficient to proceed beyond the preliminary investigation.

Acidy Cheese

The limit to which lactic acid may be allowed to develop in cheddar cheese is fixed by the isoelectric point of casein. Too much acid results in a crumbly texture and bleached or acid-cut colour in cheese.

In comparative vats of pooled milk which was partially homogenized, one vat was so manipulated as to make acidy cheese while the other was made normally. In a series of 7 pairs of such vats only a slight advantage in flavour score was indicated in favour of the acidy cheese.

Time of Homogenization

Another experimental approach to the study of the influence of acidity on the action of milk lipase in cheddar cheese was as follows. In the first of 2 comparative vats a portion of cheese milk at 86° F., to which 3% starter had been added, was withdrawn, homogenized and immediately returned to the vat before appreciable lactic acid had had time to develop. This was vat A. Milk in the second vat received the same amount of starter at the same time as the first. However, acid was allowed to develop to 0.20%, when the same proportion of milk as in the first vat was withdrawn, homogenized and returned to the vat. This was vat B. Both vats were then set and the make proceeded with in the usual manner. The difference between the 2 comparative vats was that in vat A lipase in homogenized milk was exposed to the action of developing lactic acid for about an hour longer than in vat B.

Table 1 shows a summary of flavour scores on 9 pairs of comparative vats made from morning milk. While there appears to be some variation

TABLE 1.—DATA ON COMPARATIVE CHEESE. MORNING MILK PARTIALLY HOMOGENIZED IMMEDIATELY AFTER THE ADDITION OF STARTER AND JUST BEFORE SETTING THE VAT

Expt. No.	1st Grading		2nd Grading		3rd Grading	
	Age	Flavour score	Age	Flavour score	Age	Flavour score
	days		days		days	
1 A	17	37.0	31	38.0	45	38.5
1 B	17	36.+	31	36.0	45	37.5
2 A	15	39.0	29	37.0	43	38.0
2 B	15	37.5	29	37.5	43	38.0
3 A	22	37.0	38	37.5	50	37.5
3 B	22	37.0	38	37.0	50	37.0
4 A	10	39−	23	38.0	39	37.0
4 B	10	37.5	23	37.0	39	37.0
5 A	21	38.0	37	37.5	49	38.5
5 B	21	37.5	37	36.5	49	37.5
6 A	8	40.0	22	38.0	36	39−
6 B	8	39.5	22	38.5	36	37.5
7 A	15	38.5	29	39.5	43	39.5
7 B	15	37.5	29	38.5	43	37.0
8 A	8	39.5	22	40+	36	40.0
8 B	8	39.0	22	39−	36	38.5
9 A	15	40−	29	39.0	43	39.0
9 B	15	39.0	29	38.0	43	38.0
Aver. of A cheese		38.7		38.3		38.6
Aver. of B cheese		37.8		37.5		37.6
Difference		0.9		0.8		1.0

in the flavour score from one pair of cheese to the next, the A cheese received a higher flavour score than the corresponding B cheese. For the entire series, the A cheese scored 0.9 points higher at the first, 0.8 at the second and 1.0 at the third grading than the B cheese.

In order to test whether milk lipase is attenuated on storage, milk 48 hr. old was used for a second series of experimental cheese. Other details were the same as before. The results are summarized in Table 2.

TABLE 2.—DATA ON COMPARATIVE CHEESE. MILK 48 HR. OLD PARTIALLY HOMOGENIZED IMMEDIATELY AFTER THE ADDITION OF STARTER AND JUST BEFORE SETTING THE VAT

Expt. No.	1st Grading		2nd Grading		3rd Grading	
	Age	Flavour score	Age	Flavour score	Age	Flavour score
	days		days		days	
1 A	8	39.0	22	40.5	36	40.0
1 B	8	39.5	22	37.5	36	38.0
2 A	15	39.5	29	38.0	43	38.5
2 B	15	38.5	29	38.0	43	37.5
3 A	10	39.0	24	39.0	38	38.5
3 B	10	39.5	24	38.0	38	38.0
4 A	17	39.5	31	39.5	45	38.0
4 B	17	38.0	31	37.5	45	37.5
5 A	10	39.5	24	40.0	38	39.0
5 B	10	39.5	24	40.0	38	39.0
6 A	9	39.0	23	39+	37	38.5
6 B	9	38.5	23	39.0	37	37.0
7 A	15	39.5	29	40.0	42	37.5
7 B	15	39.5	29	39.0	42	37.0
8 A	21	41.0	34	40.5	49	39.5
8 B	21	39.0	34	37.5	49	38.5
9 A	10	39.5	28	39.5	43	38.0
9 B	10	38.5	28	38.0	43	39.0
Aver. of A cheese		39.4		39.6		38.6
Aver. of B cheese		38.9		38.3		37.9
Difference		0.5		1.3		0.7

Again, cheese made from milk which was homogenized immediately after the addition of starter scored higher for flavour than identical cheese made from the same milk but which was homogenized after acidity had been allowed to develop to 0.20% in terms of lactic acid. For the whole series the A cheese scored 0.5 points higher on flavour at the first, 1.3 points at the second and 0.7 points at the third grading than the comparative B cheese.

Lactic Acid Development

Experimental procedure was once again varied so as to study the influence of lactic acid developing, over a prolonged period, or more rapidly. Pooled milk was, therefore, divided equally into 2 vats. A small amount of starter from 36 to 90 ml., was added to vat N. Both milks were held at 60° F. overnight. In the morning the vats were heated up to 86° F., 3% starter was added to vat M and, if necessary, up to 1% additional

starter to vat N. Lipase activity was induced in both vats by homogenization, as before. Cheese was made in the usual way, keeping the conditions in both vats as much alike as possible.

From the grading figures summarized in Table 3 it may be seen that the differences between the N and M comparative cheese are even greater than previously obtained. In one instance cheese made from milk to which a small amount of starter was added the night before scored 3 points higher on flavour than the corresponding cheese made from the same milk to which 3% starter was added in the morning. On the average the N cheese scored 1.0, 1.5 and 1.0 points higher at the first, second and third grading successively.

TABLE 3.—DATA ON COMPARATIVE CHEESE. MILK AT 60° F. INOCULATED WITH A SMALL AMOUNT OF STARTER AT NIGHT, AND WITH 3% STARTER IN THE MORNING

Expt. No.	1st Grading		2nd Grading		3rd Grading	
	Age	Flavour score	Age	Flavour score	Age	Flavour score
	days		days		days	
1 N	10	39.5	24	40.0	38	40.0
1 M	10	38.5	24	37.5	38	38.0
2 N	8	39.5	22	39.0	36	38.5
2 M	8	38.0	22	38.5	36	38.0
3 N	16	38.0	32	39+	44	39.0
3 M	16	37.5	32	38.5	44	37.0
4 N	14	38.5	30	39.5	42	39.0
4 M	14	37.5	30	37.5	42	38.0
5 N	14	39.5	28	39.0	43	39.0
5 M	14	37.5	28	37.0	43	38.5
6 N	7	39+	21	39.5	36	39.5
6 M	7	39.0	21	39−	36	38.5
7 N	7	39.5	22	40.0	38	39.0
7 M	7	39−	22	38.5	38	39+
8 N	15	39−	29	40.0	43	40.0
8 M	15	39.5	29	39−	43	39−
9 N	14	38.5	29	40.0	43	39.5
9 M	14	37.0	29	37.0	43	39.0
10 N	22	38.0	36	39−	50	38:0
10 M	22	37.0	36	38.0	50	37.5
11 N	8	40.0	22	40.0	36	39.5
11 M	8	38.0	22	38.5	36	37.5
Aver. of N cheese		39.0		39.5		39.2
Aver. of M cheese		38.0		38.0		38.2
Difference		1.0		1.5		1.0

DISCUSSION

At first, the experiments which have just been described were conceived in terms of straightforward inhibition of milk lipase by acid. Simultaneously with this work, however, studies on cheese fat acidity were conducted (7). Unfortunately this phase of the work was not sufficiently advanced to include data on fat acid degrees for all cheese considered in this communication. But such information later became available for most of the cheese listed in Table 3 thus supplementing the original point of view.

As reported elsewhere (7) several interesting features were revealed by studies on fat acidities. Fat acid degrees of cheese made from milk in which some acid was developed overnight were only slightly lower than that of comparative cheese made from milk to which starter was added in the morning only. More significant was the relatively high fat acidity in both. For cheese in Table 3, at least, it is indicated that the higher flavour score was not so much due to the suppression of lipase action as to a selective hydrolysis or a displacement of equilibrium in favour of non-volatile fatty acids. Work has already been cited to indicate that the course of lipase may be modified under certain conditions.

Reference has also been made to the different behaviour of milk lipase in homogenized and non-homogenized milk (5). The differences in the flavour scores between cheese made from milk in which lipase activity was induced by homogenization immediately after the addition of starter in one case and just prior to setting in another, are further evidence of this. Lipase in homogenized milk appeared to be more susceptible to the influence of lactic acid. However, the development of acid in untreated milk overnight, as in experiments in Table 3, also influenced the action of milk lipase when milk was homogenized subsequently indicating that lipase in normal milk is similarly affected. It is the time of contact of milk lipase with acid which appears to be an important factor under the conditions studied.

To what extent the improvement in the flavour score of experimental cheese described was due to inhibition of lipase and to what extent to a modification of its course of action cannot be assessed from the data available. No doubt both are involved. However, the inhibition of lipase action did not appear to be as definite as might have been expected on the basis of published results. A likely explanation is to be found in the mildly acid conditions employed in our experiments. It is interesting, therefore, that under these marginal conditions a selective influence becomes apparent. On this basis a good flavour score together with a relatively high fat acid degree in the same cheese can be reconciled.

SUMMARY

Attempts to inhibit the action of milk lipase by the addition of hydrochloric acid to milk, a portion of which was homogenized, or by making acidy cheese were only partly successful.

In comparative vats cheese milk was partially homogenized; in one vat immediately upon the addition of starter and in the other just before setting. Cheese made according to the first procedure scored higher for flavour by about one point.

In another set of experiments, cheese milk in one vat was inoculated with a small amount of starter at night and held at 60° F. while the same milk in a second vat received 3% starter in the morning. Milk lipase activity was induced in both vats by homogenization. Cheese from the first vat scored more than a point higher for flavour.

The results are explained on the basis of lipase inhibition by contact with acid over a period of time, as well as by influence of acid or a set of conditions on the course of lipase action so as to produce mainly non-volatile acids. Lipase in homogenized milk appeared to be more susceptible to the influence of acidity than lipase in normal milk.

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